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LYMPHOID TUMORS IN MICE RECEIVING ESTROGENS

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The occurrence of mammary and uterine tumors in mice receiving estrogens has been attributed, at least in part, to the specific hyperplasia of the genital tissues occurring under such stimulation. The origin of spindle cell sarcoma at or near the site of injection indicates that estrogen might act on nongenital mesenchymal tissues much as the synthetic carcinogens do.[1] The incidence of such tumors is low, however, and the significance of the observations may be questioned.[2]

Lymphoid tumors frequently occur spontaneously in certain strains of mice. In other strains the incidence is low. Such tumors developed in 3 of 111 mice of three different strains following injections of estrogen, while such tumors did not appear among the controls.[3] One of these tumors was definitely leukemic.[4] Two others arose as mediastinal masses and invaded the surrounding tissues. Similar tumors were observed in 14 mice from several different strains given estrogens.[5] Such tumors did not appear in untreated mice of these strains.

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2. Gardner, W. U.: Arch. Path. **27**:138, 1939. Burns, Suntzeff and Loeb.[1c]

3. Gardner, W. U., in Some Fundamental Aspects of the Cancer Problem: Symposium Sponsored by the Section on Medical Sciences of the American Association for the Advancement of Science, New York, Science Press, 1937, p. 67; Science, 1937, supp. 4, p. 67.

4. Lawrence, J. H., and Gardner, W. U.: Am. J. Cancer **33**:112, 1938.

5. Lacassagne, A.: Compt. rend. Soc. de biol. **126**:193, 1938.

MATERIALS AND METHODS

Mice of the C_3H strain were used in the present investigation. This strain had been inbred by brother to sister matings for at least thirty-six generations at the time young mice were removed for this study.[6] The control mice were from unselected representatives of the same generations. The treated animals include those of all groups from which the majority have been submitted to autopsy at this time and which have not been previously reported on.

The mice were maintained on a diet of Purina fox chow. At the start of each experiment 6 mice were usually placed in each cage. Food and water were available at all times.

Estradiol dipropionate, estradiol benzoate, equilin benzoate, cholesterol, cholesterol benzoate, carotene, testosterone propionate, testosterone propionate and estradiol benzoate[7] given simultaneously, and sesame oil given alone were administered to variable numbers of mice (tables 1 and 2). The chemicals administered, with the exception of colchicine, were dissolved in sesame oil. All injections were made subcutaneously on the dorsal part of the body. The amount of material given at each injection was usually 0.05 cc. The injections were started in mice 3 to 140 days of age and continued at biweekly, weekly, fortnightly or longer intervals throughout life or in some cases for limited periods of six to ten weeks.

OBSERVATIONS

Three hundred and fifty-two mice of the C_3H strain have been observed in the experiments reported here. Of these, 149 received estrogens in variable amounts throughout their lives, starting at ages ranging from 3 to 140 days. The observations on these mice are grouped according to treatment in table 1.

Estradiol dipropionate was administered to 12 mice (6 males and 6 females), starting at 34 to 36 days of age. These animals were given weekly injections of 50 micrograms subcutaneously. All the animals lived to be 150 days of age or older. One is living at 300 days of age. Of the 11 mice which died, 7 had lymphoid tumors at ages ranging from 175 to 272 days, after a total injection of 1.2 to 1.7 mg. of estradiol dipropionate.

In all of these mice the mediastinum was extensively infiltrated with lymphoid tissue. All of them presented respiratory difficulties prior to death. A white irregular mass of tissue filled the mediastinum from the manubrium to the diaphragm, invading the intercostal muscles, the pericardium, heart muscle, lungs and in some cases the esophagus, large arteries and trachea. The tumors were confined entirely to the

6. Strong, L. C.: Genetics **20**:586, 1935.

7. The estradiol dipropionate was supplied by Dr. E. Oppenheimer, of Ciba Pharmaceutical Products, Inc. The estradiol benzoate (progynon B) and testosterone propionate were supplied by Drs. E. Schwenk and M. Gilbert, of the Schering Corporation. The equilin benzoate was supplied by Dr. A. Girard, of Paris; the cholesterol benzoate was prepared by Dr. W. Bergmann; the carotene was obtained from the British Drug Houses Ltd., London, and the cortical extract was supplied by Dr. G. F. Cartland, of the Upjohn Company.

mediastinum in 4 mice. In 1 mouse a white blood cell count made two days prior to death revealed a subnormal level of 2,400 cells per cubic millimeter. In 3 other mice the spleen and lymph nodes were also involved, and the ovaries in 2 females. Sections of the liver and genital

TABLE 1.—*Incidence of Lymphoid Tumors in C₃H Mice Receiving Estrogens*

Treatment*	Mice	Number Living	Number Dying Over 150 Days of Age	Number with Lymphoid Tumors	Age at Appearance of Lymphoid Tumors	Age Range of Mice Without Tumors	Amount of Material,† Micrograms
DPB	12	1	11	7	175 247 207 270 209 272 236	200-294	50
ABG	6	0	4	1	310	284-366	16.6
EBC	6	0	6	0	...	189-445	100
EB	16	0	16	4	478 316 387 306	191-478	100
B	16	0	13	1	345	167-504	33.3
B	25	5	19	1	300	145-645	33.3/4
B	12	0	12	1	517	205-576	16.6/4
B	6	0	6	0	...	319-572	16.6/3
B	10	0	10	0	...	237-479	16.6/2
B	13	0	13	1	380	220-528	8.3
B	17	0	17	2	272 290	239-392	16.6
Colchicine B	10	0	9	4	487 444 412 346	153-467	(25 colchicine) times 11 16.6
Totals	149	6	136	22			

* DPB = estradiol dipropionate; ABG = carotene and estradiol benzoate; EBC = equilin benzoate and extract of adrenal cortex; EB = equilin benzoate; B = estradiol benzoate.

† The amount stated was given to each mouse weekly unless written as 33.3/4, which means 33.3 micrograms every fourth week.

tissues of the aforementioned 3 mice revealed general lymphoid invasion of all organs. Blood cell counts were not made immediately before autopsy in these cases. The bone marrow was almost entirely replaced by compact bone as occurs in mice receiving estrogens, so that the extent of myeloid invasion could not be determined.

Six mice were given weekly injections of 0.5 mg. of carotene and 16.6 micrograms of estradiol benzoate. Four of these mice lived for

150 days or more. One at autopsy showed enlargement of all lymph nodes. The liver, spleen and other tissues studied showed lymphoid infiltration.

Six mice were given weekly injections of 100 micrograms of equilin benzoate and 0.05 cc. of an active extract of adrenal cortex daily for one hundred and eighty-nine to four hundred and forty-five days. In none of these mice did lymphoid tumors develop. Lymphoid tumors developed in 4 of 16 mice given weekly injections of 0.1 mg. of equilin benzoate weekly for periods of one hundred and ninety-one to four hundred and seventy-eight days. Blood counts were not made on any of these mice, but all showed extensive lymphoid involvement of the mediastinum, lymph nodes and spleen. Lymphoid cells had also infiltrated other tissues, particularly the liver and kidneys. Splenic and mediastinal tissues from 2 of these mice were grafted into other mice of the same strains; the lymphoid tumors which developed were carried for many generations.[8]

Of 16 mice given 33.3 micrograms of estradiol benzoate weekly, 13 lived to be 150 days old or older. In 1 male mouse lymphoid leukemia developed, and the mouse was killed at 345 days of age. Only 2 mice in the group lived to an age of 345 days (347 and 504). In a mouse which received an injection of 33.3 micrograms of estradiol benzoate every fourth week a mammary tumor developed at 266 days of age, and the mouse when killed at 309 days of age showed marked enlargement of the spleen and lymph nodes. Study of dry imprints of these tissues stained with May-Grünwald-Giemsa stain and of the sectioned tissues proved the presence of a lymphoid tumor. Eighteen other mice, 17 of which lived to 150 days or more, died without showing lymphoid tumors. In another mouse given 16.6 micrograms of estradiol benzoate every fourth week a lymphoid tumor developed at 517 days of age, while in 11 other animals living to from 205 to 576 days of age no such tumors developed.

In none of 16 mice which received 16.6 micrograms of estradiol benzoate every second or third week did lymphoid tumors develop though they lived to from 237 to 572 days of age. Ten of the 16 mice died with mammary tumors.

One of 13 mice given 8.3 micrograms of estradiol benzoate weekly died with a lymphoid tumor at 360 days of age. A mammary tumor had developed in this mouse at 267 days of age. It was removed and recurred but did not reappear following a second operation. At autopsy a large invasive mediastinal tumor was found. The spleen and the mesenteric and other abdominal nodes were all greatly enlarged.

8. Lits, F.; Kirschbaum, A., and Strong, L. C.: Am. J. Cancer **34**:196, 1938.

In 6 of 27 mice treated with 16.6 micrograms of estradiol benzoate weekly lymphoid tumors developed. Four of 10 mice which showed tumors received eleven weekly injections of 25 micrograms of colchicine in aqueous solution in addition to the estrogen. These tumors showed the same general range of structure as those in the preceding groups. One was transplanted and maintained for two generations.

Incidence of Leukemia in Control Mice of the C_3H Strain.—Included in the group listed as control mice were untreated male and

TABLE 2.—*Incidence of Lymphoid Tumors in Untreated Mice of the C_3H Strain and in Mice Not Treated with Estrogens, Treated with Lower Amounts of Estrogen or Given Estrogen and Androgen Simultaneously*

Treatment*	Mice	Number Living	Number Dying Over 150 Days of Age	Number with Lymphoid Tumors	Age at Appearance of Lymphoid Tumors	Age Range of Nonleukemic Mice	Amount of Material, Milligrams (TP); or Micrograms (B)
TP	11	0	11	0	...	257-479	1.25 2.5
TPO	17	0	17	1	492	181-533	16.6-33.3 B 1.25-2.5 TP
OTP	30	12	18	0	...	173-461	0.5-1.0 TP 8.3-33.3 B
A	6	0	6	0	...	233-520	
B	12	0	12	0	...	227-284	$\frac{33.3\dagger}{2 \times 6}$
B	6	0	6	0	...	274-509	$\frac{33.3}{3 \times 3}$
B	9	0	9	0	...	165-299	3.3
B	12	0	12	..	...	228-567	0.033
C	10	0	10	0	...	297-618	0.5 mg.
CB	12	0	12	0	...	152-646	0.5 mg.
C_3H breeders	40	0	40	0	...	208-577	
OB	38	0	38	0	...	247-644	0.05 cc.
Total	203	12	191	1			

* TP = testosterone propionate; TPO and OTP = testosterone propionate plus estrogen; A = carotene; B = estradiol benzoate; C = cholesterol; CB = cholesterol benzoate; OB = sesame oil.

† $\frac{33.3}{2 \times 6}$ means 33.3 micrograms of estradiol benzoate every second week for six injections, after which treatment was stopped.

female mice which had been used as breeders, mice which had received weekly injections of sesame oil, cholesterol, cholesterol benzoate or carotene, injections of estrogens in low amounts or briefly, or injections of testosterone or of testosterone and estrogens simultaneously. These groups consist of all of those in which one half or more of the mice have been examined post mortem at the time of writing (table 2).

Lymphoid tumors were not observed in any of the 117 mice which had received injections of the nonestrogenic substances or had been left untreated. In none of the 39 mice which had received weekly injec-

tions of 3.33 micrograms of estradiol benzoate or less or short term injections of larger amounts of estrogens did lymphoid tumors develop. A lymphoid tumor developed in 1 of 47 mice which received simultaneous injections of estradiol benzoate and testosterone propionate. This mouse had received weekly injections of 16.6 micrograms of estradiol benzoate and 2.5 mg. of testosterone propionate for four hundred and thirty-one days. Injections were started at the age of 61 days. The mouse died with a large cervical or vaginal tumor. All subcutaneous and abdominal nodes were enlarged and white. Histologic study and an examination of dry imprints of the nodes revealed a well developed lymphoid neoplasm.

COMMENT

Lymphoid tumors may occur in mice, with leukemic involvement of the blood stream or as localized overgrowths of lymphoid tissue with or without diffuse metastases or general involvement of all lymphoid tissues. Three factors have been observed to increase the incidence of such tumors or to be associated with such an increase; namely, a genetic factor,[9] roentgen irradiation[10] and the carcinogenic chemical methylcholanthrene.[11] Other factors have likewise been claimed by various investigators to increase the incidence of lymphoid tumors.[12]

Among many strains of mice lymphoid tumors appear to a limited extent. The incidence in the C_3H strain used in this investigation appears to be very low, however. Considering the 117 untreated or nonestrogen-treated mice reported on, the incidence is quite insignificant. Lymphoid tumors have occurred in mice of this strain, but the incidence appears to be less than 1 per cent.[13] The presence of 22 such tumors among 149 mice receiving estrogens, or in 15.4 per cent, suggests that the treatment received by these animals had some stimulating effect on the appearance of lymphomatosis. The incidence of lymphomatosis and myelosis in mice subjected to roentgen irradiation was 23.5 per cent, but that of the untreated control mice studied by Furth and Furth[10] was 6.2 per cent. Though based on smaller groups of estrogen-treated mice, the increased incidence of lymphoid neoplasms in the mice reported here (0-15.4 per cent) appears of comparable significance. Myelosis, however, was not observed in mice of the C_3H strain.

The estrogens have been associated with the development of tumors of the genital tissues, it is assumed, by specific growth-stimulating effects on such tissues. The mesenchymal hemopoietic tissues have not been

9. Richter, M. N., and MacDowell, E. C.: Physiol. Rev. **15**:509, 1935.
10. Furth, J., and Furth, O. B.: Am. J. Cancer **28**:54, 1936.
11. Morton, J. J., and Mider, G. B.: Science **87**:327, 1938.
12. Richter, M. N.: Leucemia, in Downey, H.: Handbook of Hematology New York, Paul B. Hoeber, Inc., 1938, chap. 42. Richter and MacDowell.[9]
13. Strong, L. C.: Unpublished data.

generally assumed to be markedly affected by the "sex hormones." The incidence of lymphatic leukemia, though transmitted to a greater extent by the female than the male, shows no tendency toward limitation to that sex as does the incidence of mammary tumor.[9]

Certain recent experiments, however, demonstrated that estrogens affect the myeloid tissue of the bone marrow in dogs [14] and chickens.[15] In the former species agranulocytosis developed and finally anemia, which invariably terminated in death. Similar changes occurred in certain mice receiving estrogens in large amounts.[16] Also, in mice as in birds, the bone marrow became largely replaced by osseous tissue.[17a,b,c] Whether any of these changes can be associated with the development of lymphoid tumors is, however, problematic. Myelogenous leukemias were not observed. In mice bony changes were prevented by simultaneous administration of adequate amounts of testosterone propionate.[17d] One mouse so treated in this series presented a lymphoid tumor.

Malignant lymphomatosis occurred in all strains of mice which tolerated estrogens in large amounts in our laboratory. The number of mice tested and the number of control mice on which observations were completed in several other strains are inadequate at this time to warrant definite conclusions other than that the tendency described in this paper apparently is not limited to strain.

SUMMARY

Twenty-two mice (15.4 per cent) of the C_3H strain showed lymphoid tumors, leukemic or nonleukemic, among a group of 149 which had received estrogens in variable amounts starting at ages of 3 to 140 days and continuing until death.

None of 117 untreated or nonestrogen-treated mice of this same strain showed lymphoid tumors. A lymphoid tumor developed in 1 of 86 mice receiving brief estrogenic treatment or estrogen and testosterone propionate simultaneously.

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14. (*a*) Tislowitz, R.: Acta brev. Neerland. **13**:183, 1938. (*b*) Gardner, W. U., and others: Unpublished data.

15. Landauer and others: Unpublished data.

16. Unpublished data.

17. (*a*) Gardner, W. U., and Pfeiffer, C. A.: Proc. Soc. Exper. Biol. & Med. **37**:678, 1938; (*b*) **38**:599, 1938. (*c*) Pfeiffer, C. A., and Gardner, W. U.: Endocrinology **23**:485, 1938. (*d*) Landauer, W.; Pfeiffer, C. A.; Gardner, W. U., and Mann, E. B.: Proc. Soc. Exper. Biol. & Med. **41**:80, 1939.

PRODUCTION OF INTERNAL TUMORS WITH CHEMICAL CARCINOGENS

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The carcinogenic activity of various chemicals has usually been studied on skin or on subcutaneous tissue, but neoplasms have also been induced in internal organs with these agents. The internal tumors produced may be considered in three groups, according to the methods employed. The first group includes those produced by the use of carcinogenic agents which have a specific affinity for the tissues involved. Thus, aminoazotoluene when fed or injected subcutaneously produces tumors in the liver rather than at the site of application. The evidence is summarized by Shear [1] and by Cook and Kennaway.[2] Betanaphthylamine, like aminoazotoluene, produces tumors of a specific tissue, although it may be in contact with other cells at a greater concentration. Its administration, either oral or subcutaneous, results in the formation of tumors of the bladder.[3] Certain derivatives of aminoazotoluene likewise produce tumors of the bladder.[4] Although the chief site of action of aminoazotoluene is the liver, the dye also affects the thyroid, producing degenerative atrophy followed by epithelial metaplasia, which closely resembles squamous cell epithelioma.[5]

The second group of internal tumors are those induced by carcinogenic chemicals which are most effective at the site of application but which also produce neoplasms at distant sites that may be more or less susceptible to spontaneous development of tumor. Thus, when dibenzanthracene was injected subcutaneously into strain C mice, the

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1. Shear, M. J.: Am. J. Cancer **29**:269, 1937.

2. Cook, J. W., and Kennaway, E. L.: Am. J. Cancer **33**:50, 1938.

3. Hueper, W. C.: Arch. Path. **25**:856, 1938.

4. Yoshida, T.: Gann **29**:295, 1935. Otsuka, I., and Nagao, N.: ibid. **30**:561, 1936. Nagao, N.: ibid. **31**:335, 1937. Zylberszac, S.: Compt. rend. Soc. de biol. **125**:389, 1937.

5. Yoshida, T.: Tr. Jap. Path. Soc. **22**:934, 1932.

incidence of pulmonary tumors rose from "moderate" to "high"; in strain A mice it rose from "high" to "very high."[6] Tumors of the liver, also, have occasionally been produced by the subcutaneous injection of chemicals whose chief center of activity is the area of application. Andervont and Lorenz[7] observed hematomas in 8 of 17 survivors when dibenzanthracene was injected subcutaneously into 40 C_3H mice. Strong, Smith and Gardner[8] induced 8 hepatomas in 42 CBA mice by injecting 3,4,5,6-dibenzcarbazole. Boyland and Brues[9] had previously reported hypertrophic changes in the bile ducts of 80 per cent of mice receiving this agent applied to the skin. Furth and Furth[10] observed hemangioma of the liver in 2 of 96 mice when benzpyrene was injected into the spleen.

The third group of internal tumors are those produced by direct contact between the carcinogenic agent and the tissue from which the tumor arises. This method has yielded tumors in most of the tissues and with most of the carcinogenic agents tried. The available information is listed in table 1.[11] A variation of this method is of special interest. The feeding of carcinogenic hydrocarbons has resulted in occasional tumors of the upper part of the digestive tract (table 1), but tumors of the lower part of the tract have not been reported. Furthermore, some investigators have failed to observe any tumors when dibenzanthracene or benzpyrene was fed for periods of six to ten months.

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6. Andervont, H. B., cited by Fieser, L.: Am. J. Cancer **34**:37, 1938.

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9. Boyland, E., and Brues, A. M.: Proc. Roy. Soc., London, s.B **122**:429, 1937.

10. Furth, J., and Furth, O. B.: Am. J. Cancer **34**:169, 1938.

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On the other hand, the feeding of certain samples of wheat germ oil to rats has resulted in the formation of tumors of the peritoneum in from thirteen to two hundred days.[12] The oils were obtained by extrac-

TABLE 1.—*Tumors Induced by Direct Injection*

Tissue	Agent	Animals	Number with Tumor	Type of Tumor	Reference
Bone	Benzpyrene	12 mice	1	Osteosarcoma	11a
	Methylcholanthrene	33 rats	4	Fibrosarcoma	11b
Brain	Benzpyrene	11 rats	3	Hypophysial adenoma	11c
			1	Epithelial tumor of anterior lobe of pituitary	11c
	1,2,5,6-dibenzanthracene	1 rat	1	Carcinoma	11d
	Styryl 430	3 rats	3	Glioma, meningioma and glioma of ependyma	11d
Esophagus	Methylcholanthrene	50 rats	5	Rhabdomyosarcoma	11e,f
Heart	Methylcholanthrene	33 guinea pigs	1	Polymorphous cell sarcoma of auricle	11g
Kidney	Dibenzanthracene	7 rats	2	Epidermoid carcinoma	20
		51 mice	10		
Liver	Dibenzanthracene	56 mice	2	Carcinoma ?	20
Lung	Road tar dust	100 mice	61	Adenoma and adenocarcinoma	11h
	Coal smoke soot	100 mice	8	Carcinoma	11i
Peritoneum	Benzpyrene	27 mice	19	Polymorphous and spindle cell sarcoma	11j
	Methylcholanthrene	40 mice	9	Spindle cell sarcoma	11k
	Dibenzanthracene	80 mice	5	Spindle cell sarcoma	11l
	Dibenzanthracene	20 mice	5	Sarcoma	11m
		38 rats	1		
	Dibenzanthracene	10 rats	8	Spindle cell sarcoma	11n
	Thorium dioxide	10 rats	4	Fibrosarcoma	11o
Prostate	Benzpyrene	50 rats	37	Squamous cell epithelioma	19
			4	Sarcoma	19
Spleen	Methylcholanthrene	1 mouse	1	Fibrosarcoma	20
	Benzpyrene	96 mice	2	Sarcoma	10
	Benzpyrene	96 mice	2	Hemangioma	10
Stomach	Methylcholanthrene (fed)	48 mice	2	Papilloma	11p
	Benzpyrene (fed)	6 mice	5	Squamous cell carcinoma	11q
	Tar (painted on mouth)	50 rats	17	Papilloma of fore stomach	11r
	Tar, wool fat, aniline and toluenediamine	10 rats	1	Carcinoma	11s
Testis	Zinc chloride	1 chicken	Small per cent	Teratoma	11t
Trachea	Methylcholanthrene	50 rats	8	Sarcoma	11e,f
Uterus	Dibenzanthracene	21 mice	1	Epidermoid carcinoma	20

12. Rowntree, L. G.; Steinberg, A.; Dorrance, G., and Ciccone, E. F.: Am. J. Cancer **31**:359, 1937.

tion of the wheat germ with ether. Purified wheat germ oils were devoid of carcinogenic activity. Carruthers [13] and Halter [14] were unable to produce tumors by feeding wheat germ oil.

The present report deals with the action of carcinogenic agents on the spleen, liver, submaxillary glands, testis, epididymis, uterus and bone marrow and the submucosa of the stomach and duodenum. Furthermore, an attempt was made to confirm the production of tumors of the peritoneum by the feeding of wheat germ oil.

PROCEDURE

Both mice and rats were used in these experiments. The mice were males of strains A, C and C_3H, and were 8 to 10 weeks of age. The rats were 13 to 15 weeks of age. The organs to be studied were exposed surgically and either 3,4-benzpyrene or 1,2,5,6-dibenzanthracene was injected. Solutions of the carcinogenic agents in corn oil were used: 10 mg. per cubic centimeter of benzpyrene or 5 mg. per cubic centimeter of dibenzanthracene. The injections were made deeply with a narrow gage needle to minimize leakage on withdrawal. Usually only one injection was made, but in a few instances a second was given. The exact doses injected into the various organs are listed in table 2.

Injections into the bone marrow were made at weekly intervals for eighteen weeks. For this purpose a hole slightly smaller than a no. 25 needle was drilled through the upper third of the tibia. From 0.05 to 0.10 cc. of the carcinogenic solution was injected at one time. Immediately after withdrawal of the needle the opening was plugged with bone wax to prevent leakage. One tibia received an injection one week, the other the next, thus allowing two weeks between injections into any one bone. Control rats received similar injections of corn oil. Complete blood counts were made before the introduction of the carcinogenic agent and at weekly intervals for four months; thereafter examinations of the blood were made at monthly intervals.

The effect of wheat germ oil in the diet on the production of tumors was studied as follows: Fifty young adult male mice of strain A were divided into two groups of 25 each. One group received a diet consisting of 93 parts of Steenbock stock ration plus 7 parts of wheat germ oil; the other group received the same diet with 0.05 per cent aminoazotoluene added. In addition, 6 male rats 2 to 3 months of age received a diet containing 75 parts of Steenbock stock diet and 25 parts of wheat germ oil. The wheat germ oil was prepared by extracting wheat germ with ethyl ether in a continuous extractor and evaporating the ether under reduced pressure. The residue was fed without further purification.

RESULTS OF THE INJECTION OF HYDROCARBONS INTO VARIOUS TISSUES

Bone Marrow.—Although as much as 13 mg. of benzpyrene and 7.25 mg. of dibenzanthracene were injected into the bone marrow, the animals remained in good physical condition. The marrow in the long bones, however, became markedly hyperplastic. In 3 of 4 rats living

13. Carruthers, C.: Proc. Soc. Exper. Biol. & Med. **40**:107, 1939.

14. Halter, C. R.: Proc. Soc. Exper. Biol. & Med. **40**:257, 1939.

over forty weeks hard firm tumors developed at the site of injection. Two had received benzpyrene; the other, dibenzanthracene. The histologic appearance of the tumors differed somewhat, but all were classified

TABLE 2.—*Injection of Carcinogenic Agents into Various Tissues*

Animals Used	Strain	Organ	Agent Injected	Total Amount, Mg.	Tumor Noted	Length of Life After Injection, Mo.	Number and Type of Tumors
9 rats	Stock	Liver	1,2,5,6-dibenzanthracene	1.25-2.62	None	9-17	
9 rats	Stock	Spleen	1,2,5,6-dibenzanthracene	1-4	None*	9-17	
13 rats	Stock	Testis	1,2,5,6-dibenzanthracene	1-3	None	9-17	
6 rats	Stock	Epididymis	1,2,5,6-dibenzanthracene	1	None	9-15	
3 rats	Stock	Submaxillary gland	1,2,5,6-dibenzanthracene	1-1.5	1 at 15 mo. 1 to 16 mo.	12-16	2 spindle cell sarcomas
5 rats	Stock	Bone marrow	1,2,5,6-dibenzanthracene	1.75-7.25	1 at 12 mo.	4-13	1 fibrosarcoma
5 rats	Stock	Bone marrow	3,4-benzpyrene	9.5-13	2 at 9 mo.	4-11	2 fibrosarcomas
5 rats	Stock	Submucosa of stomach	3,4-benzpyrene	1.5-2	1 at 7 mo. 1 at 13 mo. 1 at 15 mo.	7-16	1 spindle cell sarcoma 1 myoma 1 adenocarcinoma
2 rats	Stock	Submucosa of duodenum	3,4-benzpyrene	1.5-2	None	13-17	
4 rats	Stock	Uterine horn	3,4-benzpyrene	1-2	1 at 12 mo.	9-16	1 myogenic sarcoma
2 rats	Stock	Spleen	3,4-benzpyrene	1.5	None	7 & 17	
1 rat	Stock	Submaxillary gland	3,4-benzpyrene	1	None	14	
3 mice	C3H	Liver	1,2,5,6-dibenzanthracene	0.25	None	6-14	
2 mice	C3H	Spleen	1,2,5,6-dibenzanthracene	0.25	1 at 6 mo.	6	1 spindle cell sarcoma
3 mice	C3H	Testis	3,4-benzpyrene	0.2	None	6	
2 mice	C3H	Submaxillary gland	3,4-benzpyrene	0.5	1 at 5½ mo.	6½	1 spindle cell sarcoma
9 mice	"A"	Spleen	3,4-benzpyrene	0.3-1.3	None	7-14	
8 mice	"A"	Testis	3,4-benzpyrene	0.3-0.5	None	4- 9	
15 mice	"A"	Submaxillary gland	3,4-benzpyrene	0.3-0.7	10 at 2-3½ mo.	4- 7	10 squamous cell carcinomas
4 mice	"C"	Submaxillary gland	3,4-benzpyrene	0.3-0.5	2 at 3 mo.	3- 6	2 squamous cell carcinomas

* One spindle cell sarcoma of subcutaneous tissue of the back occurred at sixteen months.

as periosteal fibrosarcoma (fig. 1). The tumors were invasive and grew to a large size (one weighed 75 Gm. at autopsy), but no metastases were found.

The blood picture was unchanged except for occasional slight increases in the number of lymphocytes, due possibly to mild infections. The blood count remained remarkably constant. This result differs

sharply from that of workers who have observed changes in the blood stream due to the administration of carcinogenic agents. Barnes and Furth [15] and Furth and Furth [10] observed leukemia following injections of benzpyrene into the spleens of mice. Lanza [16] observed leukemia in rats after benzpyrene was injected into the bone marrow. Erythroleukemia has been reported in 50 per cent of a series of rats receiving

Fig. 1.—Periosteal fibrosarcoma induced by injections of benzpyrene into the bone marrow of a rat.

injections of tar in the bone marrow.[17] Changes occurred in twenty to sixty days, and, once initiated, proceeded in the absence of further injections. Changes in the blood resembling leukemia have also been

15. Barnes, W. A., and Furth, J.: Am. J. Cancer **30**:75, 1937.

16. Lanza, G.: Pathologica **30**:185, 1938.

17. Bernard, J.: Sang **8**:28, 1934.

induced in mice by subcutaneous injections of sodium 1,2,5,6-dibenzanthracene-9-10-endoalphabetasuccinate.[18]

There is thus abundant evidence that carcinogenic agents do produce changes in the blood stream. Possibly our animals were unusually resistant to leukemic changes, since they actually received larger amounts of carcinogenic agents than were employed by other investigators. Moreover, since some strains of animals exhibit a much higher incidence of spontaneous leukemia than others, it is quite likely that the tendency toward induced leukemia also varies with the strain.

Submaxillary Gland.—Squamous cell carcinoma of the submaxillary gland developed in 10 of 15 strain A mice and in 2 of 4 strain C mice given injections of benzpyrene. This organ showed more rapid development of tumor than any other tissue in the body. The tumors were palpable in two to three and one-half months and attained diameters up to 2.5 cm. They were usually invasive and sometimes soft and cystic. The first stage of tumor development was a metaplasia of the glandular tissue into a stratified squamous epithelium, which then became malignant (fig. 2). The changes in the submaxillary gland parallel exactly the metaplastic and neoplastic changes which occurred in the prostate when benzpyrene was injected into that organ.[19] The submaxillary tumors were readily transplanted into animals of the same strain and to date have gone through five transplantations. In this connection it is of interest to note that squamous cell carcinoma of the skin is usually difficult to transplant. A spindle cell sarcoma was found five and one-half months after the injection of benzpyrene into the submaxillary gland of a mouse of the C_3H strain; and 2 of 3 rats given injections of dibenzanthracene likewise showed spindle cell sarcomas in fifteen to sixteen months. It appears that the rapid rate of tumor formation in this gland is restricted to neoplasms of epithelial origin.

Stomach and Duodenum.—In this experiment benzpyrene was injected into the submucosa of the pyloric region of the stomach in 5 rats and into the duodenum in 2. In 3 of the rats receiving injections in the stomach tumors developed; those receiving injections in the duodenum did not show development of tumors. The first tumor was noted at seven months, when a rat died of starvation. Autopsy revealed an obstruction in the lumen of the stomach, 3 by 3.5 cm. in diameter. It was a myoma. The second tumor was found in a rat killed after thirteen months. It was a spindle cell sarcoma, 1 by 1.5 by 2 cm., in

18. Parsons, L. D.: J. Path. & Bact. **43**:1, 1936. Burrows, H., and Cook, J. W.: Am. J. Cancer **27**:267, 1936.

19. Moore, R. A., and Melchionna, R. H.: Am. J. Cancer **30**:731, 1937.

the pyloric end of the stomach. The third tumor was a nodule 1 by 1 cm. in the pyloric region of a rat which died after fifteen months. The tumor was a low grade adenocarcinoma which had invaded the muscularis. No metastases were found in any of the animals.

Uterus.—Of 4 rats receiving injections of benzpyrene in the uterine wall, 1 subsequently showed a large myogenic sarcoma of the uterine horn

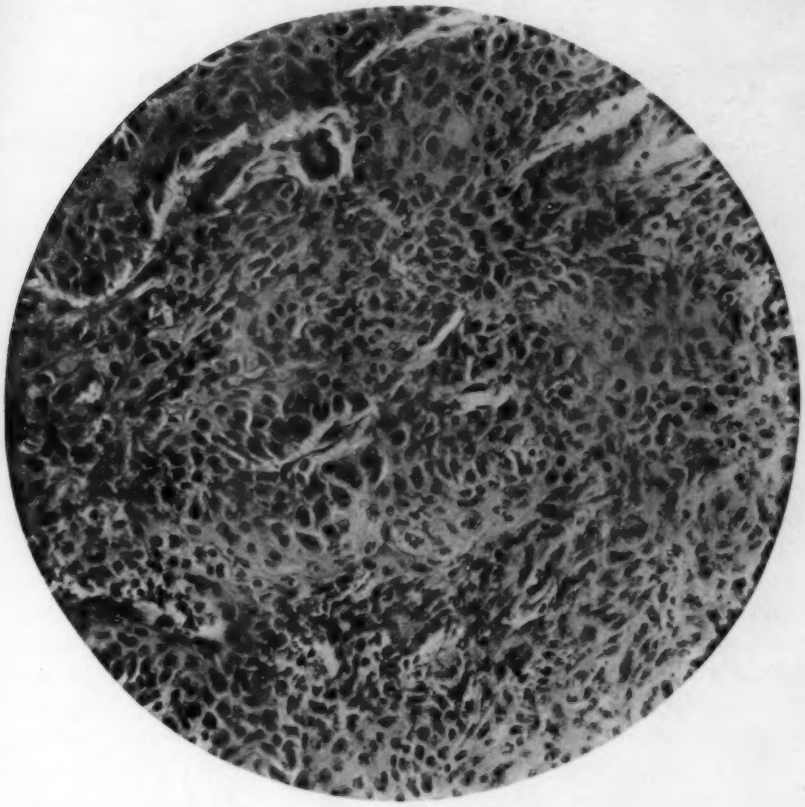

Fig. 2.—Squamous cell carcinoma induced by an injection of benzpyrene into the submaxillary gland of a mouse.

(fig. 3). The animal died twelve months after the injection of benzpyrene.

Spleen and Liver.—Of the animals receiving intrasplenic injections of benzpyrene or dibenzanthracene, 11 rats and 11 mice lived longer than five months. One only, a C_3H mouse treated with dibenzanthracene, showed development of a tumor—a spindle cell sarcoma. There were 9 rats and 3 mice that received injections of dibenzanthracene

in the liver. They survived for periods of six to seventeen months but failed to present tumors.

Testis and Epididymis.—Large amounts of the hydrocarbons (table 2) were present in the testes of both rats and mice for periods longer than necessary for the production of tumors in other tissues. Nevertheless, no tumors developed. This confirms the result of Ilfeld [20] and

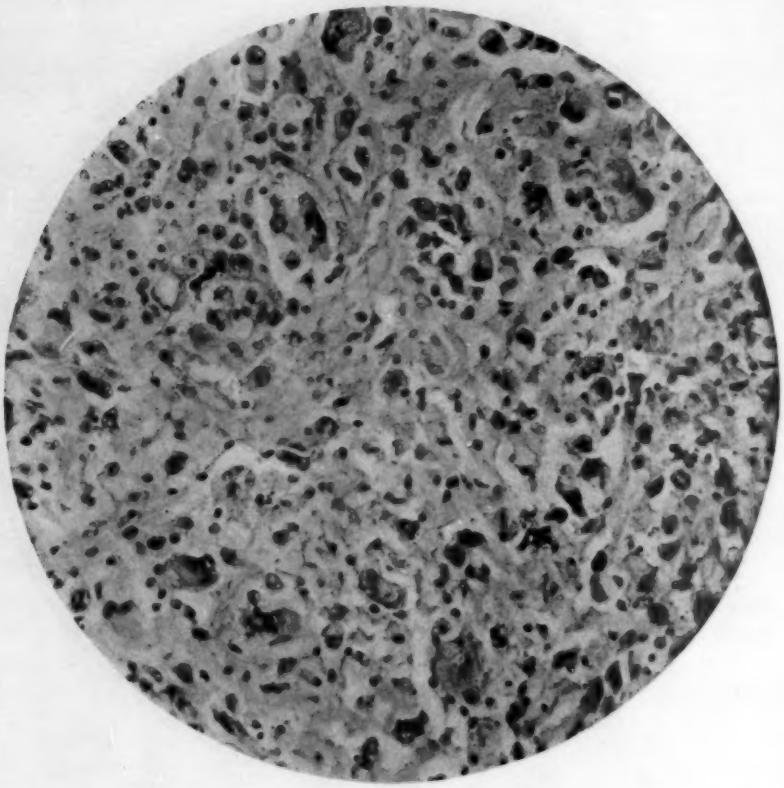

Fig. 3.—Myogenic sarcoma induced by an injection of benzpyrene into the uterus of a rat.

of Tuchmann and Demay.[21] The local toxicity of the hydrocarbons produced marked parenchymal degeneration; but no other response to the chemicals appeared. Apparently both the testes and the epididymes of rats and mice are more refractory to the action of carcinogenic hydrocarbons than are other tissues.

20. Ilfeld, F. W.: Am. J. Cancer **26**:743, 1936.

21. Tuchmann, H., and Demay, M.: Compt. rend. Soc. de biol. **123**:686, 1936.

Skin.—In 1 rat a raw area developed in the lumbar region of the back about fifteen months after the injection of dibenzanthracene into the spleen and into each testis, a total of 3 mg. being administered. One month after the development of the raw area the animal was killed. The area was 2 by 2.5 cm. in diameter and had firm raised edges. It was a spindle cell sarcoma which invaded the neighboring muscle tissue. It probably represented a tumor arising at an injured site in an animal loaded with a carcinogenic agent. A similar spontaneous tumor has never been found in our rat colony..

Results of the Feeding of Wheat Germ Oil.—Both rats and mice on the wheat germ oil diets remained in good health. No neoplasms developed. On the diet containing 7 per cent wheat germ oil, 5 mice died within three months, while 10 survived for six to ten months, and 10 were still alive at fourteen months. Survival was much poorer when aminoazotoluene was added to the wheat germ oil; 7 died within four months and the remaining 18 died in six to eight months. The rats were killed after receiving 25 per cent wheat germ oil for four months. No abnormalities of any kind were noted, although Rowntree and associates [12] reported tumors occurring within six weeks. We were therefore unable to confirm the report that wheat germ oil causes peritoneal tumors.

COMMENT

It is evident that there is a marked difference in the response of various animal cells to carcinogenic agents, since tumors develop in some tissues much more readily than in others. The standard tissues for experimental production of tumors are the cutaneous and subcutaneous tissues. When benzpyrene was injected subcutaneously into 200 of our mice of the C strain, it took five to five and one-half months for 50 per cent of the animals to show development of tumors. When benzpyrene was applied to the skin in benzene solution, the time necessary for tumors to develop in 50 per cent of the animals was five and one-half months, both in 420 "C" mice and in 160 commercial mice. This is in line with the experience of Andervont,[6] who observed no essential difference in the incidence of subcutaneous tumors of strain A and C mice following injection of dibenzanthracene, although a considerable difference does exist among several strains. In our experiments with benzpyrene the percental tumor incidence in the cutaneous and subcutaneous tissues was over 70 in all groups. In all other tissues tried except the submaxillary gland the percental tumor incidence was less than 40. The rate of tumor production also varied in the various organs. All the submaxillary tumors of epithelial origin developed within three and one-half months, as compared with five and one-half months for

50 per cent of the cutaneous or subcutaneous tumors. In the other tissues tumors were produced more slowly if at all.

The reasons for this irregularity are not apparent. Conceivably one might associate rapid development of tumor with an adequate supply of blood, but this certainty is not the dominant factor, since the supply of blood to the skin and subcutaneous tissue is appreciably less than that to the liver and testis. Nor does there appear to be a connection between the rate of tumor formation and the rate of cellular proliferation in various tissues. The skin, which is proliferating, shows no more rapid development of tumors than subcutaneous tissue, in which cellular division is not so active. Furthermore, proliferation is extensive in both the bone marrow and the testis, in neither of which when they were exposed to benzpyrene did tumors develop. Finally, it does not appear that such differences can be explained on the basis of a difference in the quantity or character of the connective tissue present in various organs. However, it appears that certain cells within an organ are more susceptible to neoplastic transformation than other cells although all have equal contact with the carcinogenic agent. This difference is clearly illustrated by the rapid formation of epithelial tumors in the submaxillary gland whereas only one tumor of connective tissue origin occurred and that at a much slower rate. The same holds true in the case of the prostate, as squamous cell carcinoma developed in 75 per cent and sarcoma in 5 per cent of the prostates of rats given injections of benzpyrene.[19]

The ease of formation of benzpyrene tumors in any tissue does not appear to parallel the susceptibility of that tissue to spontaneous tumor formation. Spontaneous tumors of the submaxillary gland or of subcutaneous tissue are not particularly common either in man or in the lower animals, as might be expected from the rapid response of these tissues to benzpyrene. Nor does resistance to benzpyrene indicate resistance to spontaneous tumor formation, spontaneous tumor of the testis, duodenum or liver being by no means rare. Thus, while a satisfactory explanation may be lacking, the fact remains that the response of a given tissue to a carcinogenic agent is specific both for the cells and the type of carcinogenic agent. A given result obtained under one set of carcinogenic factors does not enable one to predict the response of that tissue to another set of carcinogenic factors.

SUMMARY

The following organs of mice or rats were given injections of 3,4 benzpyrene or 1,2,5,6-dibenzanthracene: submaxillary gland, spleen, liver, testis, epididymis, uterus, stomach (submucosa), duodenum (submucosa) and bone marrow. The tumors resulting were: 12

squamous cell carcinomas and 3 spindle cell sarcomas of the submaxillary gland; 1 myoma, 1 spindle cell sarcoma and 1 adenocarcinoma of the stomach; 3 periosteal fibrosarcomas; 1 myogenic sarcoma of the uterus, and 1 spindle cell sarcoma of the spleen. The development of squamous cell carcinoma of the submaxillary gland was extremely rapid. The injection of the hydrocarbons into the bone marrow did not produce changes in the blood picture. No tumors were produced by the feeding of wheat germ oil.

DISEASE OF THE LIVER IN HYPERTHYROIDISM

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The relationship between toxic disease of the thyroid gland and concurrent damage of the liver is one which has been little recognized and, until late years, commonly ignored. An extensive review of "the historical data concerning the hepato-thyroid relation" has recently been published by Boyce and McFetridge.[1] Paul, in 1865, was the first to mention the association of hepatic cirrhosis and toxic thyroid disease, although Eden, in 1906, seems to have been the first to realize the possibility of a causal relationship.

Although numerous individual cases have been reported and references to a probable relation made, it has been only since 1933 that any detailed studies of the pathologic changes in the liver have appeared in the literature, viz., those by: Weller [2] (48 cases), 1933; Beaver and Pemberton [3] (107 cases), 1933; Rössle [4] (30 cases), 1933; Haban [5] (26 cases), 1933-1934, and Cameron and Karunaratne [6] (30 cases), 1935. The findings in these 241 cases are summarized by Cameron and Karunaratne.[6]

Various investigators have reported clinical evidence of hepatic dysfunction obtained by utilization of liver function tests. These include Maddock, Coller and Pedersen [7] (bromsulfthalein test), Hurxthal [8] (blood cholesterol determination), Rowe [9] (various tests), Ragins [10] (Takata-Ara test) and Althausen and Wever [11] (galactose tolerance test). A recent editorial in *The Journal of the American Medical Asso-*

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1. Boyce, F. F., and McFetridge, E. M.: Arch. Surg. **37**:427, 1938.
2. Weller, C. V.: Ann. Int. Med. **7**:543, 1933.
3. Beaver, D. C., and Pemberton, J. D.: Ann. Int. Med. **7**:687, 1933.
4. Rössle, R.: Virchows Arch. f. path. Anat. **291**:1, 1933.
5. Haban, G.: Beitr. z. path. Anat. u. z. allg. Path. **92**:88, 1933.
6. Cameron, G. R., and Karunaratne, W. A. E.: J. Path. & Bact. **41**:267, 1935.
7. Maddock, W. G.; Coller, F. A., and Pedersen, S.: West. J. Surg. **44**:513, 1936.
8. Hurxthal, L. M.: Arch. Int. Med. **52**:86, 1933.
9. Rowe, A. W.: Endocrinology **12**:1, 1928.
10. Ragins, A. B.: J. Lab. & Clin. Med. **20**:902, 1935.
11. Althausen, T. L., and Wever, G. K.: J. Clin. Investigation **16**:257, 1937.

ciation [12] called attention to the work done by Bartels and by Boyce and McFetridge utilizing the Quick hippuric acid test for determining the amount of hepatic injury in thyrotoxic patients.

There is by no means universal acceptance of the concept of hepatic insufficiency occurring as a result of hyperthyroidism. Means [13] found scant evidence supporting such a concept and classified the hepatic injury as a complication rather than as a characteristic of the disease. Mallory, quoted by Means,[14] suggested that the thyroid hormone itself does not injure the liver but that in certain cases hyperthyroidism may lead to hepatic dysfunction indirectly by increasing the vulnerability of that organ to noxious agents.

Experimentally it has been repeatedly shown that the feeding of desiccated thyroid reduces the glycogen content of the liver in a large variety of animal species. Farrant [15] demonstrated "fatty degeneration" of the liver in thyroid-fed rabbits and cats, while Gerlei [16] observed necrosis in the centers of liver lobules in rabbits five to seven days after administering lethal doses of thyroxin subcutaneously. Hashimoto [17] produced parenchymatous changes in the liver and in the convoluted tubules of the kidneys of albino rats by feeding thyroid substance. However, Youmans and Warfield [18] failed to note such changes in dogs given large amounts of thyroid extract, nor did liver function tests show any evidences of dysfunction.

Mention should also be made of accumulating evidence that vitamin deficiency may play a part in hepatic insufficiency. Ivy [19] expressed the belief that there is a definite relationship, direct or indirect, between the liver, vitamin B_1 and the vitamin B_2 complex, and the thyroid. He quoted Drill [20] as showing that the administration of the vitamin B_2 complex protects the liver from the deglycogenizing effect of thyroxin.

MATERIAL

All cases of well established clinical toxic thyroid disease in which necropsy was done at the Cincinnati General Hospital during the period from 1926 to 1938 were studied. Cases were eliminated in which there were other, independent

12. Hippuric Acid Test as an Index of Hepatic Damage, editorial, J. A. M. A. **111**:1470, 1938.

13. Means, J. H.: Thyroid and Its Diseases, Philadelphia, J. B. Lippincott Company, 1937, p. 319.

14. Means,[13] p. 292.

15. Farrant, R.: Brit. M. J. **2**:1363, 1913.

16. Gerlei, F.: Ann. d'anat. path. **10**:555, 1933.

17. Hashimoto, H.: Endocrinology **5**:579, 1921.

18. Youmans, J. B., and Warfield, L. M.: Arch. Int. Med. **37**:1, 1926.

19. Ivy, A. C.: Personal communication to the author.

20. Ivy, A. C.: Internat. Abstr. Surg. **66**:4, 1938; in Surg., Gynec. & Obst., January 1938. Drill, V. A.: J. Nutrition **14**:355, 1937.

anatomic factors which might have produced hepatic pathologic changes or in which there was uncertainty about the clinical diagnosis. A total of 24 cases was collected (table 1).

A detailed study was made of representative microscopic sections in each case. When indicated, slides were restained, or new sections were prepared from gross material preserved at necropsy.

For a careful comparative study of the pathologic observations in the liver in routine necropsy material, two series of cases were selected. The hepatic changes were studied in a group of 50 consecutive cases in which death from traumatic injuries occurred within forty-eight hours after the accident. Because of the possibility that a certain amount of hepatic change in cases of thyrotoxicosis might be secondary to passive venous congestion, analysis was made of a second group of 50 consecutive cases in which the cause or primary contributory cause of death was chronic rheumatic heart disease. Eliminated from both groups were all cases not falling within the age limits of the toxic thyroid series. No other exclusions were made.

A study of the pathologic changes in the liver in cases of vitamin B and vitamin C deficiency, occurring singly and combined, was also made to determine whether

TABLE 1.—*Clinical Diagnoses Made in Twenty-Four Cases of Hyperthyroidism*

Clinical Diagnosis	Cases	Males	Females
Toxic nodular goiter	14	1	13
Toxic diffuse goiter	6	2	4
Adenomatous goiter with secondary hyperthyroidism	3	..	3
Adenocarcinoma of the thyroid	1	..	1
Totals	24	3	21

there were any changes common to this group and cases of toxic thyroid disease. Thirty-three such cases were collected; in this group the most frequent clinical diagnosis was pellagra.

CLINICAL FINDINGS

The group of 24 thyrotoxic patients comprised 3 males and 21 females. The preponderance of the latter was higher than is usually observed and was probably due to the relatively small total number of patients. There were 18 white and 6 Negro patients. The average age was 53 years, the youngest being 27 years old and the oldest being 76 years. Included in the group were 2 persons aged 76 and 74 years, respectively. The former had been operated on sixteen years before death (bilateral superior pole ligations were performed at that time) and had suffered a recurrence of symptoms during her last year of life. The latter had an enlarged thyroid for thirty-three years with symptoms of thyrotoxicosis for slightly more than a year preceding death. Three patients were admitted in thyroid crisis. Two of these were jaundiced.

Icterus has often been described as a clinical finding in toxic thyroid disease. In this series it was present in 5 persons. In 3 of these jaundice was well marked and in 2 evident only in the scleras. For the entire series of 24 patients the basal metabolic rates ranged from + 18 to + 73 per cent, the average being + 41 per cent. The duration of symptoms averaged slightly less than two years. Iodine therapy was instituted in 67 per cent, the average duration of treatment being eleven days. No liver function tests were performed. In 2 recorded cases the

icteric index was +36 and +75. Two patients had marked pitting edema of the lower extremities and reversal of the albumin-globulin ratio. A history of alcoholism was obtained from 3 patients. One of these admitted moderate indulgence; the other 2 were definitely addicts. Diabetes mellitus was an associated condition in 2 patients. The Wassermann reactions of the blood of 3 patients were positive.

PATHOLOGIC OBSERVATIONS

A reduction of liver weight in fatal cases of hyperthyroidism has been mentioned by various authors. Beaver and Pemberton [3] stated that loss of weight of the liver was possibly the most important hepatic change which they found, and gave 1,316 Gm. as the average weight in their series. In the present series of 24 cases of toxic thyroid disease the average weight of the liver was found to be 1,275 Gm. (table 2). The average weight found in 50 cases of chronic rheumatic heart disease

TABLE 2.—*Incidence of Common Pathologic Changes of the Liver in Various Reported Series of Fatal Cases of Hyperthyroidism*

Series	Cases	Average Weight of Liver, Gm.	Percentage of Cases with Given Hepatic Change		
			Fatty Infiltration	Chronic Localized Interstitial Hepatitis (Including Cirrhosis)	Cirrhosis (All Stages)
Rössle (1933)*	30	1,225	....	....	....
Haban (1933)*	26	.....	23.0	....	38.5
Weller (1933)	48	.....	"Frequently noted"	87.5	....
Beaver and Pemberton (1933)	107	1,316	87.8	59.8	14.9
Cameron and Karunaratne (1935)	30	1,157	80.6	....	33.3
Present series	24	1,275	91.7	83.3	25.0

* The series of Rössle and of Haban are not complete.

was 1,600 Gm., while in 50 cases of accidental death it was 1,570 Gm. It is true that loss of body weight is a constant finding in hyperthyroidism and that the weight of the liver should perhaps be computed as percentage of body weight. Nevertheless, just as loss of general weight is accepted as a clinical manifestation of the disease, loss of hepatic weight should be regarded as a pathologic change.

Fatty infiltration of varying degrees is commonly observed in the liver. Probably the two factors of greatest importance in causing this are intoxication and insufficient oxygenation. Canzanelli and Rapport [21] found that the consumption of oxygen by the guinea pig liver was depressed when thyroid tissue was incubated with it. This led them to suspect the presence of a substance in the thyroid inhibiting oxygenation.

21. Canzanelli, A., and Rapport, D.: Endocrinology **22**:73, 1938.

Connor [22] expressed the belief that chronic severe fatty infiltration of the liver progresses in many cases to "fatty cirrhosis." With Chaikoff and Biskind [23] he recently demonstrated that cirrhosis develops following severe fatty infiltration in depancreatinized diabetic dogs. Fatty infiltration occurred in all but 2 of my series of 24 cases of toxic thyroid disease. The picture was that commonly seen in fatty metamorphosis of the liver. Care was taken to use the same standards throughout, not only in judging this change but in judging all pathologic changes noted in the liver. Independent observations were made by another observer and the results carefully checked. The degree or intensity of the changes was specified by three descriptive terms—"mild," "moderate" and "marked" (table 3). Of the 50 cases of accidental death, a diagnosis of alcoholism was made in 4. Probably the incidence of alcoholism would have been much higher had it not been for the fact that a considerable proportion of the patients died before regaining

TABLE 3.—*Incidence of Fatty Infiltration of the Liver in a Series of Cases of Hyperthyroidism and in Control Series*

Series	Cases	Number Showing Given Degree of Fatty Infiltration			
		Mild	Moderate	Marked	Total
Toxic thyroid disease	24	6	9	7	22
Chronic rheumatic heart disease	50	12	10	3	25
Accidental death	50	15	10	6	31

consciousness, so that no history was obtained. Fatty infiltration of the liver was observed in 62 per cent of the cases in this group. It was encountered in 50 per cent of the cases of chronic rheumatic heart disease and was almost invariably associated with severe chronic passive congestion. Attention should be drawn also to the differences in severity of this acute degenerative process in comparative studies. Moderate to markedly severe fatty changes were encountered in the liver in 67 per cent of the cases of toxic thyroid disease, while of this range changes were observed in only 26 per cent and 32 per cent of cases, respectively, of chronic rheumatic heart disease and accidental death.

While fatty infiltration of the liver may perhaps be considered an acute degenerative process, the presence of a low grade inflammatory reaction at the peripheries of liver lobules, associated often with patchy increase in fibrous connective tissue and with lymphocytic infiltration, is certainly indicative of a more chronic process. It is consistently found in its

22. Connor, C. L.: J. A. M. A. **112**:387, 1939.

23. Chaikoff, I. L.; Connor, C. L., and Biskind, G. R.: Am. J. Path. **14**:101, 1938.

most marked form in true diffuse cirrhosis of the liver. This form of chronic localized interstitial hepatitis was found in 83 per cent of the 24 cases reported here (table 4). The sole criterion for making the diagnosis was lymphocytic infiltration of the periportal areas. No attempt was made to estimate changes in fibrous connective tissue except where marked periportal fibrosis or definite cirrhosis was evident. Comparative studies showed this lesion to be present in 22 and 26 per cent of cases, respectively, of accidental death and chronic rheumatic heart disease. Furthermore, the degree of change was ordinarily far more intense in the cases of thyrotoxicosis than in the control groups.

The criteria used for the diagnosis of cirrhosis were degenerative changes leading to the disappearance of hepatic cells, a chronic inflammatory reaction occurring at the peripheries of the altered lobules, and regeneration of an imperfect type, such changes being generally distributed in the liver. Cirrhosis was found in 6 of the 24 cases; it was advanced in 2 and of moderate degree in 4. In only 1 instance was it

TABLE 4.—*Incidence of Chronic Localized Interstitial Hepatitis in a Series of Cases of Hyperthyroidism and in Control Series*

Series	Cases	Number Showing Given Degree of Chronic Localized Interstitial Hepatitis			
		Mild	Moderate	Marked	Total
Toxic thyroid disease	24	10	6	4	20
Chronic rheumatic heart disease	50	8	4	1	13
Accidental death	50	7	3	1	11

found in the accidental death series and in no cases in the chronic rheumatic heart disease series. During the years 1935 and 1936, in 1,431 consecutive necropsies the diagnosis was made sixty-nine times, an incidence of 4.7 per cent. In the thyroid series there were 3 cases in which periportal lymphocytic infiltration was associated with patchy fibrosis. The possibility exists that this is an early stage in the development of cirrhosis.

It is difficult to evaluate the incidence of cirrhosis in this as compared with other reported series because of the dissimilarities in diagnostic criteria. Most observers have classified increased periportal fibrosis with the more specific, easily recognized cirrhoses and have called it by various terms, such as "insular cirrhosis," "early chronic atrophic cirrhosis" and "patchy chronic parenchymatous interlobular hepatitis" (table 2). Weller expressed the belief that cirrhosis of the liver in hyperthyroidism occurs only as an advanced stage of chronic interlobular hepatitis. Hahan, quoted by Bartels,[24] suggested the name "cirrhosis

24. Bartels, E. C.: Ann. Int. Med. **12**:652, 1938.

Basedowiana" for the condition of those livers found in fatal cases of toxic thyroid disease which show any chronic lesions of a cirrhotic type.

Hepatic lesions secondary to chronic venous stasis were observed in 9 of the cases of thyrotoxicosis. The lesions most frequently encountered were dilatation of venous sinusoids, atrophy of liver cords and varying degrees of necrosis of the central zones. A careful check on these changes was provided by the series of fatal cases of chronic rheumatic heart disease, in which well marked hepatic lesions secondary to chronic passive congestion were found in 80 per cent of the cases. Well marked hepatic fibrosis was observed in 9 of the 50 cases of chronic rheumatic heart disease; however, this increase in connective tissue in all cases originated from the centers of the lobules instead of appearing in the periportal regions and was usually most marked in the subcapsular lobules. There was no evidence of associated chronic interstitial

TABLE 5.—*Incidence of Common Hepatic Lesions in a Series of Cases of Deficiency of Vitamin B_1, the Vitamin B_2 Complex and Vitamin C as Compared with a Series of Cases of Hyperthyroidism*

		Number Showing Given Degree of Fatty Infiltration				Number Showing Given Degree of Chronic Interstitial Hepatitis				
Series	Cases	Mild	Moderate	Marked	Total	Mild	Moderate	Marked	Total	Cirrhosis
Vitamin B and C deficiencies	33	14	8	11	33	14	6	1	21	0
Toxic thyroid disease	24	6	9	7	22	10	6	4	20	6

hepatitis. The lesion observed was that sometimes referred to as cardiac cirrhosis.

Microscopic study was made of the livers of 33 patients whose death was caused directly or indirectly by deficiency of vitamin C, vitamin B or the vitamin B_2 complex. There were histories of alcoholism in 14 of these patients. Although fatty infiltration was evident in every liver, the degree of involvement was more than moderate or marked in only 57 per cent as contrasted with an incidence of 67 per cent in the toxic thyroid series. Well marked chronic interstitial hepatitis was found in only 20 per cent and cirrhosis in none of these livers (table 5).

Microscopic sections of the thyroid gland were reviewed in every case. There was marked variation in the pathologic changes not only between sections from different glands but also between sections from the same gland. Since iodine therapy had been instituted in the majority of the cases, varying degrees of involution were seen. In 19 of the 24 cases there was definite evidence of epithelial hyperplasia. Associated with this proliferation of epithelial cells there were observed varying amounts of colloid, usually poorly stained and vacuolated. An increased

number of newly formed blood vessels and lymphocytic infiltration in the interacinar tissue were also noted in almost every one of these 19 cases. In 4 of the 24 cases marked involutionary changes were present, with occasional epithelial spurs extending into the acini. The colloid was well stained and the epithelium lining the acini flattened. In each of these 4 cases, however, lymphocytic hyperplasia in the interstitial tissue was a constant finding. The involutionary changes were believed to be due either to intensive iodine therapy or to a remission of the hyperthyroidism. In 1 of the 24 cases a typical picture of adenocarcinoma was observed.

ASSOCIATED CHANGES OBSERVED AT NECROPSY

The possibility that pathologic changes may occur in the kidneys secondary to degenerative hepatic changes has been advanced by numerous observers. The term "hepatorenal syndrome" has frequently been applied to the association, and various theories have been advanced to explain clinically evident renal insufficiency or abnormal urinary findings in the presence of hepatic disease. Many investigators who have produced hepatic lesions by feeding thyroid substance have reported associated renal changes, which they have ascribed solely to the induced hyperthyroidism. Thus Farrant [15] describes, in addition to "fatty degeneration" of the liver in thyroid-fed cats and rabbits, changes in the kidneys suggestive of "tubular nephritis." Both Hashimoto [17] and Goodpasture [25] described parenchymatous changes in the convoluted tubules of the kidneys as well as in the livers of thyroid-fed albino rats. However, Bartels [26] found no disturbance in renal function as tested by the urea clearance test in 25 cases of hyperthyroidism.

In the present study, marked degenerative changes of the renal convoluted tubules were noted in association with advanced cirrhosis of the liver in 1 case. Extensive cytoplasmic degeneration of the tubular epithelium was evident, and with the scarlet red staining technic in combination with study under the polarizing microscope it was possible to demonstrate large amounts of doubly refractile lipid in the epithelial cells. There were no glomerular changes to support a diagnosis of glomerulonephritis. In 4 other cases tubular degenerative changes of lesser degree were noted, in 1 of which they were associated with cirrhosis of the liver. Vacuolization of the epithelium of the loops of Henle was seen in the 2 cases complicated by diabetes mellitus.

Focal degeneration of the myocardium, often described in fatal cases of hyperthyroidism, was not noted in any case. Changes in the adrenal

25. Goodpasture, E. W.: J. A. M. A. **76**:1545, 1921; J. Exper. Med. **34**:407, 1921.

26. Bartels, E. C.: New York State J. Med. **39**:117, 1939.

bodies consisted of cortical nodular hyperplasia in 3 cases, so-called cortical adenoma in 2 and early adenocarcinoma in 1. Hyperplasia of the thymus was observed in 6 cases. Leiomyoma of the uterus was found in 12 of the 20 females in the group.

COMMENT

A great deal of attention has been paid to clinical observations of hepatic insufficiency in thyrotoxic patients. Comparatively little note has been made of the pathologic changes in the liver which might produce this dysfunction. Unfortunately, no liver function tests were done on the 24 patients in this study. However, icterus occurred in 5, well marked in 2 and mild in 3. In a patient with an icteric index of + 36 the most important etiologic factor was probably extensive passive congestion, although fatty infiltration also was observed. In 3 patients jaundice was associated with cirrhosis of the liver. In the fifth patient, a young man with a clinical history of only three months' duration, who died in thyroid crisis, icterus was confined to the scleras, and the only significant hepatic change was extensive fatty infiltration.

It has been suggested by various investigators, Dinsmore [27] and Lahey [28] among others, that delirium and disorientation after thyroidectomy and even death associated with hyperthyroidism are chiefly due to hepatic failure. Certainly the almost invariable occurrence of pathologic changes in the liver in the 24 cases presented here is strong presumptive evidence of such a possibility. This is the more convincing since severe hepatic lesions, with the exception of fatty infiltration, were negligible in the control group of 50 cases in which death resulted from traumatic injuries. Except for changes secondary to chronic passive congestion, extensive alterations were found with relative infrequency also in the control group of 50 cases in which death was due to chronic rheumatic heart disease. In each of the 3 cases of hyperthyroidism in which crisis was a clinical manifestation there was moderate fatty infiltration of the liver. One of these cases showed associated chronic localized interstitial hepatitis, while another was complicated by well marked cirrhosis of the liver.

The possibility exists that too much importance has been placed on the constant finding of fatty infiltration of the liver in fatal cases of thyrotoxicosis, since such fatty change commonly occurs in many conditions other than hyperthyroidism. However, the high percentage of association together with the severity of the changes makes it impossible to ignore them. In the majority of cases a combination of several hepatic pathologic changes was noted. Probably the lesion of greatest signifi-

27. Dinsmore, R. S.: J. A. M. A. **109**:179, 1937.
28. Lahey, F. H.: New England J. Med. **213**:475, 1935.

cance was chronic interstitial hepatitis, sometimes associated with periportal fibrosis or cirrhosis. While lymphocytic infiltration at the peripheries of liver lobules without known cause is occasionally encountered in routine necropsies, the degree of involvement is usually slight.

No lesions were observed similar to those reported in the livers of animals suffering from induced hyperthyroidism. Although central necrosis of liver lobules was not infrequently encountered, in every case chronic passive congestion could be demonstrated as the etiologic factor. On the other hand, congestion appeared to play no part in the production of chronic hepatitis or cirrhosis. Experimental evidence that administration of excessive amounts of thyroid results in depletion of the glycogen in the liver and that a good store of glycogen exerts a protective influence against poisons such as chloroform has suggested the hypothesis that the hepatic lesions in hyperthyroidism are due to exhaustion of hepatic glycogen. Cameron and Karunaratne [6] found that the minimal toxic dose of carbon tetrachloride for thyroid-fed albino rats remained the same as for normal control rats. Clinically, however, Bartels [24] recently demonstrated that the use of a diet high in carbohydrate improved liver function, as indicated by the excretion of increased amounts of hippuric acid.

An attempt was made to correlate clinical and pathologic observations, but the series of cases was too small to allow complete conclusions to be drawn. Beaver and Pemberton [3] expressed the belief that the common factor in the evolution of hepatic lesions appears to be related to the severity of the syndrome of exophthalmic goiter. In the present series, the average duration of symptoms in 6 cases of thyrotoxicosis associated with cirrhosis of the liver was two and a half years; if these 6 cases are excluded, the average duration of symptoms was slightly less than two years. Lymphocytic infiltration in the periportal regions was noted in all cases in which the duration of symptoms was more than one year but in only 8 of the 12 cases in which the history was one year or less.

Two patients in whom thyroid disease was complicated by diabetes mellitus presented severe fatty infiltration of the liver. In one of these cirrhosis was also present. Cirrhosis of the liver was present in both patients for whom a diagnosis of chronic alcoholism had been made clinically in addition to that of hyperthyroidism. The role that alcohol plays in the production of cirrhosis has been a source of controversy for many years among physiologists and experimental pathologists. The majority of investigators have been opposed to the concept of alcohol as a direct cause of cirrhosis. Recently Hall and Morgan [29] pointed

29. Hall, E. M., and Morgan, W. A.: Arch. Path. **27**:674, 1939.

out that accumulating evidence indicates that for man, at least, the body and more especially the liver must be in a state of altered metabolism before cirrhosis will result from heavy drinking of alcoholic liquor. Possibly the hepatic lesions in these 2 patients might not have progressed to cirrhosis in the absence of heavy drinking. The liver of another patient, who gave a history of moderate indulgence, presented moderate fatty infiltration and mild chronic interstitial hepatitis.

SUMMARY

It is believed that the results of this study indicate a relationship between toxic disease of the thyroid gland and hepatic damage. The lesions of the liver observed in 24 fatal cases of toxic thyroid disease were loss of liver weight, fatty infiltration, cirrhosis and lymphocytic infiltration in the periportal regions, often associated with patchy fibrosis. These hepatic changes appeared with greater frequency and severity in this series of 24 cases than in a carefully studied group of 100 control cases.

The average weight of the liver in the thyrotoxic group was 1,275 Gm., as compared with 1,582 Gm. in the control group. Fatty infiltration was found in 92 per cent of the cases, while in only 56 per cent of the control group was there any evidence of this change. Evidence of a chronic inflammatory reaction was found in 83 per cent of cases of toxic thyroid disease whereas the incidence in the control series was only 24 per cent. The incidence of cirrhosis of the liver was 25 per cent in cases of hyperthyroidism and 4.7 per cent in 1,431 consecutive routine necropsies.

The hepatic lesions found in association with thyrotoxicosis cannot be said to result from passive venous congestion. Their production does not appear to be related to deficiencies of vitamins B and C. There is a possibility that the degenerative lesions of the renal tubules which occur in patients suffering from hyperthyroidism may be due directly to the thyrotoxicosis or secondarily to the hepatic changes.

Despite the limited number of cases in the present study it seems evident that more chronic lesions are found in the livers of patients with longer histories of toxic thyroid disease. The character of the pathologic changes in the liver in fatal cases of thyrotoxicosis appears sufficient to explain the clinical evidences of hepatic insufficiency.

CHEMISTRY OF ATHEROSCLEROSIS

I. LIPID AND CALCIUM CONTENT OF THE INTIMA AND OF THE MEDIA OF THE AORTA WITH AND WITHOUT ATHEROSCLEROSIS

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Numerous studies have been made in the attempt to determine the cause and importance of the lipid deposits in the lining of the aorta and of the large arteries. Most of them have been morphologic rather than chemical. Neither method has provided all the information necessary to interpret the conditions. Some authors considered the lipid deposits incidental in the evolution of atherosclerosis; others regarded them as integral factors. The first view [1] emphasized focal injuries of the media, either anatomic (Thoma; Faber) or functional (Lange; Staemmler), as the initial changes. It interpreted the intimal hyperplasia as compensatory and the fatty and other retrogressive changes as secondary or, at most, of limited importance. According to the second view [1] (Marchand; Lubarsch; Aschoff; Anitschkow; [2] Leary,[3] and others), the essential factor is the penetration of the plasma lipids into the intima. Mechanical conditions favor plasma infiltration into the regions where subsequent degenerative changes and tissue reactions occur.

Wells [4] reviewed the early chemical studies. These analyses demonstrated an increase in the lipid content of the aorta with advancing age and severity of atherosclerosis. The lipid extracts from atherosclerotic aortas were reported to contain cholesterol, "lecithin" and fatty acids. In 1926 Schönheimer [5] in a systematic chemical analysis of atherosclerotic aortas observed an increase of the total lipids and a variation in the proportions of the various lipid constituents with increasing

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1. Beitzke, H.: Virchows Arch. f. path. Anat. **267**:625, 1928.
2. Anitschkow, N.: Virchows Arch. f. path. Anat. **249**:73, 1925.
3. Leary, T.: Arch. Path. **17**:453, 1934.
4. Wells, H. G., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933.
5. Schönheimer, R.: Ztschr. f. physiol. Chem. **160**:61, 1926.

severity of atherosclerosis. The cholesterol esters varied from about 10 per cent of the total lipids in the aortas of youths to 60 per cent in those with atherosclerosis. The average amount of free cholesterol remained constant. Schönheimer [6] later reported an average of 3 per cent for the phospholipids and a progressive increase with age and degree of atherosclerosis. His phospholipid values were low, probably because of the method of extraction. He also found undetermined small amounts of fatty acids, "protagon" and a substance similar to "oxycholesterol." Neutral fats were not identified. Page and Menschick [7] found a substance having absorption bands at 238 and 320 millimicrons in the cholesterol fraction of calcified aortas. These absorption bands resemble those of cholestenone. The significance of this observation is uncertain. Cholesterol from other viscera did not contain this light-absorbing component. Schönheimer [8] confirmed its presence in extracts of atherosclerotic aortas. He was unable to detect ergosterol by spectroscopic examination, or any trace of vitamin D by biologic assay, in calcified aortic tissues.

Kimmelstiel's [9] systematic analysis of human aortas for cholesterol, phosphatides and galactosides demonstrated a proportional increase of all fractions until atheromas formed. Then the relationships changed and the cholesterol increased rapidly in comparison with the other fractions. The galactoside content reported by Kimmelstiel amounted to 1 to 1.5 per cent of the dry weight of the aorta. Lehnherr,[10] in a comparative study of aortas in diabetes and nondiabetic conditions, observed that arteriosclerosis in the aorta is associated with: (1) increased amounts of cholesterol, phospholipids and fatty acids, (2) an increased proportion of cholesterol in the total lipids, and (3) increased amounts of calcium and phosphorus with a diminished Ca/P ratio. The lipid deposits and other changes in diabetes were similar but more marked. Rosenthal's [11] analysis of 500 aortas for "fat" content confirms the previous reports that atherosclerosis is associated with an increased lipid content of the tissue. He made no attempt to determine the individual lipids. The analyses mentioned thus far were made on extracts of entire aortas.

Meeker and Jobling [12] emphasized the local distribution of the lesions in atherosclerosis and stated that an aorta may have several stages of the disease. They correlated the lipid analyses of individual lesions with

6. Schönheimer, R.: Ztschr. f. physiol. Chem. **177**:143, 1928.
7. Page, I. H., and Menschick, W.: Naturwissenschaften **18**:585, 1930.
8. Schönheimer, R.: Ztschr. f. physiol. Chem. **211**:65, 1932.
9. Kimmelstiel, P.: Virchows Arch. f. path. Anat. **282**:402, 1931.
10. Lehnherr, E. R.: New England J. Med. **208**:1307, 1933.
11. Rosenthal, S. R.: Arch. Path. **18**:473 and 606, 1934.
12. Meeker, D. R., and Jobling, J. W.: Arch. Path. **18**:252, 1934.

the character of the atherosclerotic process. With age, an increase in lipid content, especially of total cholesterol, and a progressive increase in the ratio of combined to free cholesterol were noted in normal tissues. In pathologic tissues the ratio increased, then decreased with the appearance of necrosis and calcification. The proportion of phospholipids remained constant. Zeek [13] reported the same findings.

The purpose of this study is to determine the variations of the lipid constituents and of the calcium content of the intima and of the media of the aorta with increasing age and with increasing severity of atherosclerosis. The aortas used for chemical analysis were obtained during routine postmortem examinations at St. Luke's Hospital. The adventitia and associated fat were stripped off without difficulty and discarded. The more difficult separation of the intima from the media was accomplished by placing the aorta with the intima down on a flat surface. After a diagonal cut had been made with a razor blade at one corner, the separation was started with the thumb nail, and the two coats were pulled apart. The intima was thin, translucent and without the fibrous character of the media. The fat deposits generally were limited to the intima. When the coats were separated, there was no trace on the media of the lesion in the intima. In marked atherosclerosis with extensive ulceration and necrosis, the media also was involved. The separation of the intima from the media was especially easy in aortas of advanced age.

ANALYSIS

Extractions.—Representative portions of the media from the thoracic and abdominal portions of the aorta were weighed in a stoppered bottle, then torn into strips ⅛ inch (0.32 cm.) wide, cut into fine pieces and transferred to a fat-free paper extraction thimble. The tissues were extracted continuously with boiling redistilled 95 per cent alcohol for eight to twelve hours. The thimble was washed several times with warm ether; the ether washings were added to the alcohol extract. Further extraction with alcohol and ether demonstrated only small traces of residual lipid. The alcohol extract and the ether washings were evaporated to dryness in a water bath at 60 to 70 C. The oily residue was taken up in small amounts of warm purified petroleum benzine (petroleum ether) and the liquid filtered through a fat-free paper into a 50 cc. volumetric flask. The volume was made up to 50 cc. Portions of this extract were used for the determinations of lipid.

Determinations.—(*a*) Nonlipid residue. The extraction thimble was dried to constant weight in an oven at 110 C. The dry weight of the tissues was determined by adding the weights of the lipid-free residue and the total lipids.

(*b*) Total lipid. A 10 cc. aliquot of the petroleum ether extract was pipetted into a tared porcelain crucible and placed in an incubator until the solvent had evaporated. The crucible was dried to constant weight in a vacuum desiccator.

(*c*) Ash content. The crucible containing the lipid residue was ignited to constant weight with a Meker burner. A few drops of nitric acid were used to remove the last traces of carbon.

13. Zeek, P. M.: Am. J. Path. **12**:115, 1936.

(*d*) Cholesterol. A portion of the petroleum ether extract was diluted with a 50 per cent mixture of alcohol and acetone until 2 cc. contained between 0.1 and 0.2 mg. of cholesterol. The free and total cholesterol were determined by a slight modification of the Schönheimer and Sperry[14] method. The color readings were made with the Sanford-Sheard[15] "photelometer." Duplicate analyses checked within 5 per cent.

(*e*) Phosphatide. Five cubic centimeter portions of the petroleum ether extract were pipetted into graduated 15 cc. centrifuge tubes and the solvent was evaporated to 1 cc. by immersing the tubes in hot water a few minutes. Eight cubic centimeters of acetone was added and the phosphatides precipitated by the Bloor[16] method. The ether-soluble fraction (lecithin plus cephalin) was separated from the ether-insoluble fraction according to Kirk.[17] The phosphorus was determined in each fraction by the Fiske and Subbarow[18] method except that the color readings were made photoelectrically.

(*f*) Galactoside. Hydrolysis was carried out on 5 cc. aliquots of the extract by Kirk's[19] method and galactose determined by the ceric sulfate titration method of Miller and Van Slyke.[20] As the reducing power of galactose is not the same as that of dextrose, the ceric sulfate was standardized against the former sugar. The reducing power of galactose was 80 per cent of dextrose, a result in agreement with the 83 per cent reported by Giragossintz, Davidson and Kirk.[21] Strict proportionality was observed over wide ranges of concentration.

(*g*) Fatty acids. No direct method exists, unfortunately, for the determination of free fatty acids in lipid extracts. As free fatty acids are only a small percentage of the total lipid, a large error is introduced when the calculation is made by difference between the total and the phospholipid and cholesterol fatty acids. The percentage of residual lipid is probably a fair index of the fatty acid content, since Schönheimer[8] showed the absence of glycerides in significant quantities.

(*h*) Calcium. A portion of the lipid-free residue was weighed in a porcelain crucible, then ashed. Several treatments with nitric acid oxidized traces of the organic matter. The ash was determined, the residue treated with a little hydrochloric acid and diluted to a definite volume, and the calcium in an aliquot determined by the Clark and Collip[22] method.

RESULTS

Media.—The variations in the lipid constituents of the media with advancing age are given in table 1. Although all these tissues appeared normal in the gross, microscopic examination disclosed that the media of the aged tissues, especially those with large plaques, had lipid deposits.

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14. Schönheimer, R., and Sperry, W. M.: J. Biol. Chem. **106**:745, 1934.
15. Sanford, A. H., and Sheard, C.: J. Lab. & Clin. Med. **15**:483, 1930.
16. Bloor, W. R.: J. Biol. Chem. **82**:273, 1929.
17. Kirk, E.: J. Biol. Chem. **123**:623, 1938.
18. Fiske, C. H., and Subbarow, Y.: J. Biol. Chem. **66**:375, 1925.
19. Kirk, E.: J. Biol. Chem. **123**:613, 1938.
20. Miller, B. F., and Van Slyke, D. D.: J. Biol. Chem. **114**:583, 1936.
21. Giragossintz, G.; Davidson, C., and Kirk, P. L.: Mikrochemie **21**:21, 1937.
22. Clark, E. P., and Collip, J. B.: J. Biol. Chem. **63**:461, 1925.

Despite individual variations, there is a definite increase in the total lipid content of the media with advancing age. The increase is general for all lipids studied except the lecithin-cephalin fraction. The trends can be seen better in table 2, where the mediae have been divided into three age groups. The free cholesterol in the youngest age group is about

TABLE 1.—*Variation in Composition of Media with Age*

				Cholesterol†			Phosphatide†				Nonlipid Residue	
No.	Age	Moisture*	Lipid Extract†	Free	Total	Ester	Ether Soluble	Ether Insoluble	Total	Galactoside†	Ash†	Calcium†
1	12	66.7	5.94	0.66	0.94	0.47	....	....	1.64	0.85	....	....
2	22	74.5	6.29	1.22	1.54	0.54	2.96	0	2.96	0.50	2.12	0.21
3	23	57.4	4.08	0.89	1.70	1.37	1.64	0.32	1.96	0.26	....	....
4	27	53.0	6.08	0.90	1.90	1.09	1.57	0.11	1.68	0.60	1.92	0.40
5	28	72.5	7.50	1.45	3.38	3.26	2.06	0.41	2.47	0.33	1.83	0.19
6	38	68.4	6.30	1.01	1.14	0.22	1.92	0.36	2.28	0.67	2.56	0.51
7	43	67.5	9.55	1.77	3.19	2.40	2.06	0.67	2.73	0.46	4.71	1.23
8	45	69.0	9.18	1.32	1.76	0.74	2.27	1.35	3.62	1.01	3.05	0.70
9	49	75.4	9.71	1.41	2.08	1.13	1.00	1.26	2.26	2.12	5.75	1.30
10	49	72.5	5.77	1.16	1.84	1.15	1.46	0.33	1.79	0.99	4.38	1.10
11	51	74.3	7.82	1.68	3.32	2.78	1.99	0.33	2.32	0.76	....	....
12	52	69.5	7.75	1.21	2.84	1.90	0.80	1.42	2.22	0.90	4.32	1.15
13	53	71.8	6.53	1.05	1.44	0.66	....	....	2.15	0.93	....	....
14	53	71.4	8.08	1.68	2.33	1.10	0.96	3.18	4.14	1.00	4.69	1.13
15	58	74.8	10.40	1.32	2.88	2.63	0.20	2.40	2.60	1.35	7.67	....
16	65	71.3	10.40	2.49	3.70	2.04	....	....	3.38	0.71	9.52	3.12
17	64	74.9	8.22	1.50	2.82	2.22	....	....	....	....	....	....
18	67	66.1	10.60	1.62	3.74	3.57	1.62	0.89	2.51	1.56	....	....
19	67	71.7	11.00	1.65	3.71	3.47	0.79	2.26	3.05	0.90	....	2.57
20	68	72.5	9.72	1.85	3.11	2.12	2.16	0	2.16	0.68	4.69	1.36
21	71	67.4	10.90	2.42	5.40	5.02	0.78	2.42	3.20	0.43	11.30	3.50
22	72	74.2	11.00	1.75	4.43	4.52	1.56	1.89	3.45	1.07	7.58	2.26
23	75	66.3	9.10	1.76	3.56	3.06	1.27	0.76	2.03	0.50	8.35	2.38
24	78	70.2	11.80	1.72	3.89	3.66	0.53	2.52	3.05	1.42	9.28	2.94
25	84	68.7	13.00	1.98	4.00	3.40	1.62	1.74	3.36	1.24	7.06	2.22

* The content is given as percentage of wet tissue.
† The content is given as percentage of dry tissue.

TABLE 2.—*Variation in Composition of Media by Age Groups*

			Cholesterol†			Phosphatide†				Nonlipid Residue	
Age Group	Moisture*	Lipid Extract†	Free	Total	Ester	Ether Soluble	Ether Insoluble	Total	Galactoside†	Ash†	Calcium†
0-40	68.4	6.42	1.05	1.78	1.24	2.13	0.22	2.21	0.59	2.11	0.33
41-60	71.8	8.31	1.36	2.23	1.44	1.25	1.53	2.70	1.09	4.90	1.10
61-84	70.3	10.57	1.87	3.84	3.90	1.29	1.59	2.91	0.95	8.25	2.57

* The average content is given as percentage of wet tissue.
† The average content is given as percentage of dry tissue.

1 per cent of the dry weight of the media. The cholesterol ester is slightly higher, the ratio being about 1.2. In the middle age group both values increase slightly, but the ratio 1.1 remains about the same. In the third age group the values for free and combined cholesterol increase greatly, the former by about 40 per cent, the latter by 130 per cent. The ratio thus increases markedly to about 1.8.

The phosphatides in the mediae of group 1 are 2.21 per cent of the dry weight with the lecithin-cephalin proportion about 90 per cent of the total. Group 2 has an increase of 22 per cent of total phosphatide over group 1, due to the increase (600 per cent) in the sphyngomyelin (ether-insoluble fraction), which greatly exceeds the decrease in the ether-soluble phosphatide of 41 per cent. In both phosphatide fractions there is a further slight increase in the highest age group, but the proportions remain essentially the same. The significance of these phosphatide values is unknown. The galactoside values parallel those of sphyngomyelin. Too much significance should not be given these galactoside values, however, because of the inaccuracies inherent in the method of analysis. Though somewhat lower than those reported previously,[20] they are probably closer to the actual values.[19]

The percentage of unidentified lipid material increases slightly with age. The exact composition of this fraction is unknown, but a large proportion probably is free fatty acids.

The lipid values of the first two age groups undoubtedly represent values for normal tissue. Histologically, stainable lipid is absent. The mediae of the highest age group have some deposits microscopically; grossly, there was no evidence of such deposits. These lipids appeared microscopically as diffuse bands lying between, but not involving, the elastic fibers. Such medial involvement appeared only under necrotic intimal lesions, especially in the abdominal portion, and was confined to the layers adjacent to the intima.

Of the nonlipid constituents, the moisture content remained relatively constant. Individual variations were great, probably because of evaporation during preparation. There was no marked trend with age. The calcium content of the media, low in young aortas, advanced regularly with age to 2.57 per cent of the dry weight in the oldest. This increase in calcium was without demonstrable microscopic change. The calcium content in general paralleled the total ash content.

Intima.—The intimas chosen for analysis were free from gross lipid deposits. Microscopically, however, there were occasional foam cells, but the small number of these in comparison with the total tissue had no great significance. The large amount of lipid in the intima as compared with the media of the same aorta was remarkable. The calcium content of the intima was low in comparison with that of the media.

Summary.—The results indicate that an accumulation of lipids, including both cholesterol and phospholipids, occurs in the aging process of the aorta. The increase is most marked in the cholesterol esters and sphyngomyelin. There is no definite correlation between these increases in the media and the type of lesion in the intima.

The increase of calcium in the media with age seems to indicate a normal physiologic process without causal relation to, and probably not significant in, calcification of the intima. This appears clearly in a comparison of the calcium content of the media with the condition in the corresponding intima. For example, in the middle age group there are six media tissues, nos. 7, 8, 9, 10, 12 and 14, with about the same calcium content. In two of these, 8 and 12, there were scarcely any changes of the intima; in nos. 7, 10 and 12 there was moderate atherosclerosis; in no. 9 there was severe atherosclerosis with extensive calcification. The lack of correlation between intimal and medial calcification is even more obvious in the highest age group. Nos. 21 and 25 contained the lowest amounts of calcium; the corresponding intimas had marked calcification. No. 16, very high in calcium, had a practically normal intima without calcification and with only slight fatty changes.

TABLE 3.—*Variation in Composition of Intima with Age*

					Cholesterol†			Phosphatide†				Nonlipid Residue	
No.	Age	Moisture*	Lipid Extract†	Free	Total	Ester	Ether Soluble	Ether Insoluble	Total	Galactoside†	Ash†	Calcium†
6	38	70.9	9.44	....	....	....	1.44	0	1.44	1.66	1.61	0
10	49	75.8	13.6	1.83	3.01	1.99	2.56	0.33	2.92	1.59	2.06	0.26
12	52	61.8	18.7	3.56	8.40	8.12	0.53	2.94	3.47	0.72	2.37	0.47
14	53	76.1	14.5	2.37	7.70	8.98	....	....	3.11	0.55	1.80	0.20
17	64	73.2	15.7	1.27	4.58	5.57	1.90	0.32	2.31	0.37	2.13	0.18
Average		71.6	14.4	2.26	5.92	6.16	1.63	0.90	2.65	0.98	1.90	0.23

* The content is given as percentage of wet tissue.
† The content is given as percentage of dry tissue.

Though not enough normal intimas have been analyzed to permit generalizations as to the variation of lipid with age, intimal tissue has a relatively high lipid content and a low calcium content as compared with media.

The water content of media, in spite of large individual variation, has no significant variation with age. This observation does not support the hypothesis of Wells [4] that arteriosclerosis arises from an inability to hold the constituents in solution as a result of the dehydration of the elastic tissue with age.

The results recorded in the preceding paragraphs disclose with advancing age an accumulation of lipids and calcium in the media independent of the atherosclerosis in the intima. Analyses of intimal lesions in atherosclerotic aortas may be summarized as follows: The lesions were separated as much as possible from the normal medial and intimal tissues and classified as fatty, fibrous and calcified plaques and atheromatous ulcers. The first type was the streaked or nodular yellow deposit, in which the lipid material was mainly intracellular and the intima was

slightly thickened but not appreciably scarred. The second type had raised nodules or diffuse plaques covered with tough fibrous material with the typical pearly luster, necrosis and pooling of lipids. The third type consisted of brittle calcified plaques with centers of soft necrotic material, characteristic of these late lesions. The atheromatous ulcers were ruptured or at the stage of incipient rupture, and contained, in addition to the soft necrotic lipid material, clotted blood and some visible calcification. These types probably represent successive stages of the disease.

TABLE 4.—*Values of Constituents of Intimal Lesions According to Age*

					Composition of Lipid Extract								
							Phosphatide‡					Nonlipid Residue	
Type of Lesion	No.	Age	Moisture*	Lipid Extract†	Cholesterol‡ Free	Cholesterol‡ Ester	Ether Soluble	Ether Insoluble	Total	Galactoside‡	Ash‡	Ash†	Calcium†
Fatty plaques													
	2	22	77.4	25.0	14.9	32.9	....	....	30.7	2.76	6.6	2.77	0.13
	26	35	....	30.8	11.5	44.4	11.6	4.2	15.8	5.20	0	1.67	0.23
	9	49	67.8	25.7	19.0	24.1	9.1	11.5	20.6	6.91	3.7	8.87	2.22
	27	67	57.2	22.2	17.0	47.2	....	....	17.8	7.00	4.2	....	....
Fibrous plaques													
	7	43	64.9	9.55	13.0	25.9	7.2	13.1	20.3	3.73	2.91	19.5	7.15
	10	49	67.3	37.8	19.9	65.2	1.05	11.6	13.1	5.95	3.8	12.8	4.63
	19	67	66.7	37.4	16.4	51.9	3.53	10.0	13.5	3.44	....	8.28	2.56
	20	68	62.8	30.5	21.6	50.5	10.9	0	10.9	3.72	2.90	4.53	1.43
	28	78	70.9	20.6	16.0	35.5	....	....	12.8	4.50	5.2	....	....
Calcified tissues													
	15	58	48.7	14.4	18.1	50.8	1.54	14.4	15.9	3.7	3.1	....	18.9
	27	67	28.6	9.23	20.0	51.0	....	....	13.7	5.7	2.3	65.2	37.1
	20	68	45.2	14.2	20.1	54.9	5.91	0	5.91	3.92	4.52	45.9	17.7
	22	72	27.5	10.3	18.1	43.1	5.98	3.5	9.5	3.04	....	66.8	24.9
	24	78	34.1	13.8	20.6	44.1	0.88	13.1	14.0	6.40	3.96	65.0	25.0
	25	84	47.5	14.6	30.0	29.2	4.10	14.2	18.3	3.70	....	62.3	22.1
Atheromatous ulcers													
	29	51	....	33.3	23.8	42.8	6.0	10.6	16.6	5.8	0	39.2	13.8
	30	68	....	35.9	27.0	46.5	4.2	11.0	15.2	4.0	0.4	24.1	8.3
	21	71	62.1	32.4	29.8	31.5	3.70	11.7	15.4	2.70	2.91	33.1	12.3
	23	75	59.4	42.5	23.7	40.6	8.37	5.75	14.1	4.10	5.6	17.2	6.0

* The value is given as percentage of wet tissue.
† The value is given as percentage of dry tissue.
‡ The value is given as percentage of total lipid.

The results of the analyses are in tables 4 and 5. The values were determined as described for media, except that the values for the lipid constituents represent the percentage of the total lipid rather than of the dry tissue. As the disease progresses through fibrous thickening, calcification and atheroma, there is an increase of free cholesterol as well as of cholesterol esters up to a certain level; then a decrease in the latter is noted. This confirms the observations of Meeker and Jobling, who noted a decrease in the ratio of cholesterol esters to free cholesterol in the late stages. In these analyses the decrease in the ratio

is not so great. The total phospholipid remained relatively constant in the various lesions, but within this lipid class extremely wide variations were noted between the proportions of ether-soluble and ether-insoluble constituents. Here, as in the media, there was a decrease in the ether-soluble phosphatides and an increase in the sphyngomyelin fraction. The high proportion of sphyngomyelin in many of these extracts is significant because previous workers have termed this fraction lecithin. Some anomalies are noted with regard to the phospholipid fractions when different types of lesions from the same aortas are compared. For example, three tissues were analyzed from aorta 20: normal media and calcified and fibrous tissues from the intima. Ether-soluble phospholipids were absent, and the phospholipid fractions were low. The extracts from the media and intima of aorta 10 were high in ether-soluble phospholipids, whereas the fibrous plaques contained only a small pro-

TABLE 5.—*Average Values of Constituents of Intimal Lesions*

			Composition of Lipid Extract Corrected for Ash Content							
					Phosphatide‡				Fatty Acids,	
Type of Lesion	Moisture*	Lipid Extract†	Cholesterol‡ Free	Cholesterol‡ Ester	Ether Soluble	Ether Insoluble	Total	Galactoside‡	Neutral Fat, etc.‡	Calcium†
Normal intima	71.6	14.4	14.2	38.6	13.7	6.4	20.1	8.0	19.1	0.23
Early fatty plaques	67.5	25.9	16.2	38.5	10.8	8.2	19.0	5.8	20.5	0.86
Fibrous plaques	66.5	27.2	18.1	47.5	5.9	9.0	14.9	4.5	15.0	3.94
Calcified tissues	38.6	12.8	21.9	47.2	3.9	9.3	13.2	4.6	13.1	24.3
Atheromatous ulcers	60.8	36.0	27.2	42.1	5.8	10.2	16.0	4.3	10.4	10.1

* The content is given as percentage of wet tissue.
† The content is given as percentage of dry tissue.
‡ The content is given as percentage of total lipid.

portion of this fraction. The galactoside fraction varied widely and inconsistently in the lesions.

There was also a sharp decrease in the "neutral fat–fatty acid" fraction. The values for this fraction were obtained by difference; thus, its actual composition is unknown. It consists probably of free fatty acid, a small proportion of neutral fat, and some unsaponifiable matter related to cholesterol.

There were no great or abrupt changes in the lipid composition with advancing severity of the lesions. Even in extremely necrotic and calcified lesions the difference in lipid composition from normal tissue did not vary greatly from the individual variations found in extracts of lesions of the same type.

As in the normal intima, the amount of calcium found in the early deposits is small. Not until fibrous thickening and pooling of lipids occur is there an increase in calcium content. High calcium values are found thereafter, especially where visible calcification has occurred.

COMMENT

If the lipid deposits in atherosclerosis of the aorta arise through infiltration by the plasma without selective activity of the tissues, the early deposits in which little necrosis has occurred should approximate in composition the lipid portion of the blood plasma. The most comprehensive study of the lipid composition of human plasma is that of Page, Kirk, Lewis, Thompson and Van Slyke.[23] They determined free and total cholesterol and phospholipid in the petroleum ether extracts of blood plasma from normal men of ages from 20 to 101 years. The analyses, made according to the gasometric methods of Van Slyke and co-workers,[24] are the best figures available at present. The cholesterol and phosphatides were determined directly; the "neutral fat" fraction was computed by difference between the total lipids and the phosphatides, cholesterol and cholesterol esters. As cerebrosides were not determined by these authors, this fraction has been included in the neutral fat fraction in our figures to provide a better basis of comparison between the two sets of values. There is a striking agreement between these figures, as shown in table 6, despite the differences in analytic procedures.

TABLE 6.—*Comparison of Lipid Composition of Blood Plasma and Arterial Tissue*

	Blood Plasma*	Intima*	Early Plaques*	Media*
Free cholesterol	14.1	14.2	16.2	17.3
Cholesterol esters	33.3	33.6	33.5	16.7
Phospholipids	22.8	20.1	19.0	34.1
Neutral fat, etc.	23.3	27.1	26.3	31.9

* The values are given as percentages of total lipid.

In a recent article Kirk [17] reported on the distribution of the three individual phospholipids and cerebrosides in blood plasma. Among these four constituents are the same large variations we found in the tissue lipids, thus affording additional, though indirect, evidence that the latter are derived directly from the plasma.

The close agreement in composition between lipid extracts from the plasma and the normal intima and the wide differences between the lipid extracts from the latter and the media indicate that the lipids of the intima originate in the plasma rather than in the protoplasm of the cells. The main portion of the lipids of the intima, in other words, is extracellular. The foregoing comparison supports the validity of Anitschkow's hypothesis that the intima is freely permeable to the lipids

23. Page, I. H.; Kirk, E.; Lewis, W. H.; Thompson, W. R., and Van Slyke, D. D.: J. Biol. Chem. **111**:613, 1935.

24. Kirk, E.; Page, I. H., and Van Slyke, D. D.: J. Biol. Chem. **106**:203, 1934.

of the plasma and that the intima wall exerts no selective action on these lipids. The close agreement between the composition of the extracts from the normal intima and that of the early fatty deposits suggests further that these deposits arise through nonspecific deposition of the plasma lipids. The changes in lipid composition which occur with advancing severity of the disease may be the result of several simultaneous and different processes. These are phagocytosis, with other attempts of the tissues to remove a foreign substance, and necrosis resulting from altered nutrition or from chemical action of the lipid substance deposited [25] in the tissues. These processes are secondary to an initial deposition of lipid, as is borne out by the appearance of stainable lipid in the intima as the earliest change observed microscopically.

Cholesterol, the only specific lipid detected microscopically, has been stressed as the important substance in atherosclerosis. The dramatic consequences of cholesterol feeding in rabbits have emphasized further its importance. The analytic results reported here indicate, however, that not merely cholesterol but lipid substances in general are involved in atherosclerosis. They indicate that the effect of cholesterol in the production of experimental atherosclerosis is not direct but is exerted through its effect on the physicochemical state of the lipids as a whole in the plasma. This role of cholesterol in atherosclerosis may be verified by experiments in rabbits and by analyses of aortic lesions similar to those reported here. Although there is reasonable evidence that the lipids in the lesions of atherosclerosis originate in the blood plasma, the cause of their deposition remains obscure.

SUMMARY

The lipid and calcium contents of the media of the human aorta increase with age. The increase is not correlated with the degree of atherosclerosis of the intima.

Intima without lesions has larger amounts of lipid and smaller amounts of calcium than the corresponding media.

With increasing severity of the atherosclerotic lesions of the intima the proportions of free and combined cholesterol increase until the onset of necrosis; then the proportion of the combined cholesterol decreases. Also, there are increased proportions of ether-insoluble phospholipids and calcium and decreased proportions of ether-soluble phosphatides, galactosides and fatty acids.

The proportions of the individual lipid constituents in the intima and in the simple fatty deposits of the intima correspond closely with those reported for these substances in blood plasma. These relations imply that the lipid deposits in the intima are the result of nonselective infiltration and precipitation of lipids from the plasma of the blood.

25. Christianson, O. O.: Arch. Path. **27**:1011, 1939.

MORPHOGENESIS OF EXTRASKELETAL OSTEOGENIC SARCOMA AND PSEUDO-OSTEOSARCOMA

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The occasional occurrence of so-called extraskeletal osteogenic sarcoma is well known. The tendency of forms of traumatic myositis ossificans to simulate malignant osteogenic sarcoma and rarely to become such is also recognized. Extraskeletal osteogenic sarcoma not related to traumatic myositis and osteosarcoma probably originating in heterotopic bone deposits in various viscera are also familiar, and these plus a number of tumors resembling osteogenic sarcoma and readily confused with that disease are of sufficient interest to warrant an attempt at analysis of conditions under which they arise and peculiarities leading to their curious morphologic appearance.

Early medical observers recognized the rarity of osseous tumors unrelated to the bony skeleton. In 1700 Boneti [1] described an osteoma of the mammary gland. Morgagni,[2] in 1769, Astley Cooper,[3] in 1845, and Johannes Müller,[4] in 1838, described ossified tumors of the female breast. Numerous reports concerning bone and cartilaginous tumors of the mammary gland appeared during the latter part of the nineteenth century. Raso,[5] in 1937, was able to collect 75 cases of "chondro-osteoma" and "chondro-osteosarcoma" of the human mammary gland. He cited 8 cases of so-called osteogenic sarcoma of the breast which he found recorded in the literature.

Pick,[6] Funkenstein,[7] Solaro [8] and Broders and Pemberton [9] have reported cases of osteogenic sarcoma primary in the thyroid gland.

From the Memorial Hospital.

1. Boneti, T.: De ventris tumore, in Sepulchretum, sive anatomia practica excadaveribus morbo denatis, Geneva, Cramer et Perachon, 1700, vol. 3, sect. 21, obs. 61, p. 522.

2. Morgagni, J. B.: The Seats and Causes of Diseases, translated by B. Alexander, London, A. Miller and T. Cadell, 1769, vol. 3, letter L, obs. 41, p. 63.

3. Cooper, A.: The Anatomy and Diseases of the Breast, Philadelphia, Lea & Blanchard, 1845, p. 47.

4. Müller, J.: Ueber den feinern Bau der krankhaften Geschwülste, Berlin, G. Reimer, 1838, p. 48.

5. Raso, M.: Pathologica **29**:229, 1937.

6. Pick, F.: Ztschr. f. Heilk. **13**:71, 1892.

(Footnotes continued on next page)

Coley,[10] Rhoads and Blumgart,[11] Mallory [12] and others have discussed osteogenic tumors occurring in the soft parts, many of them histologically identical with osteogenic sarcoma of the bony skeleton. Most of these tumors were described as clinically benign, although often the patients were inadequately followed. Coley pointed out that a few of the osteogenic tumors which arise on the basis of traumatic myositis are clinically malignant, and the patients have pulmonary metastases. Butler and Woolley [13] reported a metastasizing osteogenic sarcoma which developed in a bulky calcified hematoma. Ewing has often mentioned osteogenic sarcoma developing in a calcific deposit of old phthisis bulbi. Heterotopic bone formation is evidently common in phthisis bulbi.

The quoted literature and other papers which for brevity's sake are not cited here offer little or nothing concerning the mechanism of the morphogenesis of these curious tumors. Thus osseous and cartilaginous tumors of the uterus, ovary, lung, kidney, pleura, meninges and other tissues are described, but their morphologic nature is unexplained.

Experimental workers in the early part of the century presented histologic evidence of metaplasia of connective tissue into bone. More recent investigations, such as those of Asami and Dock,[14] Huggins [15] and others, have stressed the concept of metaplasia of connective tissue into bone under some unknown influence. Leriche and Policard [16] recalled the old observations of von Recklinghausen on the relation of calcification to slowing of the lymphatic flow. They pointed out that in ossification following fracture the most important morphologic indication in the region to be ossified is the appearance of an edematous state, hard and lardaceous, and peculiar to connective tissue about to ossify. They noted that a gelatinous substance infiltrates the interstices of the connective tissues. They emphasized the fact that zones which may be expected to ossify soon have few vessels and they linked the aforementioned edema to defective circulation and stagnation of the connective tissue fluids. Newly formed spicules of bone are said to be situated as far as possible

7. Funkenstein, O.: Virchows Arch. f. path. Anat. **171**:34, 1903.

8. Solaro, G.: Clin. chir. **21**:1101, 1913.

9. Broders, A. C., and Pemberton, J. de J.: Surg., Gynec. & Obst. **58**:100, 1934.

10. Coley, W. B.: Ann. Surg. **57**:305, 1913.

11. Rhoads, C. P., and Blumgart, H.: Am. J. Path. **4**:363, 1928.

12. Mallory, T. B.: Am. J. Path. **9**:765, 1933.

13. Butler, F. E., and Woolley, I. M.: Radiology **26**:236, 1936.

14. Asami, G., and Dock, W.: J. Exper. Med. **32**:745, 1920.

15. Huggins, C. B.: Arch. Surg. **22**:377, 1931.

16. Leriche, R., and Policard, A.: The Normal and Pathological Physiology of Bone: Its Problems, translated by S. Moore and J. A. Key, St. Louis, C. V. Mosby Company, 1928.

from the blood capillaries which enter the nonossified connective tissue spaces. These authors likewise noted that an increase in circulation of the blood results in resorption of bone, that new bone formation may follow concomitant rarefaction of either bone or calcified elements and that rarefaction either of bone or of calcified substance may lead to new bone formation in the neighboring connective tissue. The relation of blood supply to normal calcification of bone has lately been emphasized by Blair.[17]

We are not interested in merely adding to the number of reported cases of ossified soft part tumors but rather in determining the mechanism of their morphogenesis. Study of a number of extraosseous tumors which bear at least some resemblance in certain of their areas to osteogenic sarcoma convinces us that these areas present several modes of origin.

The simplest origin is seen in the ossified basal cell carcinoma. In the examples of this tumor in our possession a metaplastic origin of the bone in the sense of true metaplastic epithelial bone cannot be seriously considered. The earliest indication of impending ossification is found in a thick hyaline deposit about capillaries and tiny arterioles. The hyaline substance is not at this stage confined to the pericapillary area but is found in the capsular connective tissue of the tumor and in the walls of major vessels of the vicinity. Whether changes appear first in the larger or in the smaller vessels cannot be determined from this material. The hyalinization about capillaries seems to lead to total obliteration, and in many hyaline strands no lumen can be identified. The process may involve a considerable area. There follows a peculiar bluish edematous degeneration of the hyaline cord, and calcific granules are deposited in this matrix. The calcific plaque is not confined to the hyaline tissue of the tumor stroma but may be found in the walls of surrounding larger vessels. Where readily identifiable vessels remain they show a definite tendency to assume a thin-walled cavernous form, possibly a dilatation following more distal obstruction through hyaline obliteration. Be that as it may, it is clear that one of the conditions for ossification and calcification in this tumor is circulatory failure caused by (1) hyaline obliteration of capillaries and (2) development of cavernous vessels favoring stasis and consequent probable anoxemia. A feature which escapes explanation is the cellularity of the residual periosseous stroma, which may approach that of a true neoplastic process and in appearance may be linked, perhaps, to active myositis ossificans. Osteoblast condensation may be noted. Numerous multinucleate giant cells testify to resorptive activity.

17. Blair, H. C.: Surg., Gynec. & Obst. **67**:413, 1938.

It is the cellularity of the interosseous stroma which causes difficulty in deciding whether the bone is formed in connective tissue or whether it is metaplastic bone in the sense that the epithelial cells of the tumor become bone cells. The separation of tumor cells and interosseous stroma is difficult. Tumor cells invade the stroma and break up into fine strands and isolated elements which when molded by surrounding spindle-shaped cells tend to assume a spindle form, and one derives the false impression that these cells are entering into bone. Ulceration, granulation tissue and infection further confuse the picture. Bone development, however, is clearly defined wherever it is seen to proceed independent of epithelium.

Such a lesion, despite its superficial resemblance to a bone-forming tumor, is of course nothing of the sort. The bone is not neoplastic bone in the usual sense. It is quite possible, however, that this sort of bone formation passes over into neoplastic osteogenesis. Indeed, we have good evidence that essentially similar mechanisms obtain in tumors which cannot be distinguished on mere morphologic grounds from osteogenic sarcoma.

An interesting combined cancer and osteogenic sarcoma was recently reported by Budd and Breslin.[18] They interpreted it as a mixed tumor because the osteogenic tissue exhibited as much evidence of invasion and rapid and autonomous growth as the carcinoma. The difficulties of interpretation of the material are evidenced by the fact that Ewing, Mallory and Masson, all of whom saw the material, reached different conclusions as to the nature of the process. Since the sections sent by Budd to Ewing are still in the Memorial Hospital collection, they have been restudied by us in connection with sections from other tumors of pertinent types, and our views coincide with those of Ewing, although his letter is not sufficiently specific to indicate his own detailed explanation of the histologic picture. The description of Budd and Breslin indicates that the tumor was one of long duration in a state of essential quiescence (twenty-eight years). It is therefore most unlikely that it was mammary cancer, for if it were, we should have to assume that mammary cancer developing in a young woman of 30 years—a period of unusual malignancy for the average mammary cancer—grew slowly for twenty-eight years. Masson and Ewing classed the tumor as mammary. Mallory believed it to be mammary, since he thought it originated in a fibroadenoma, yet Budd and Breslin did not describe it as in the breast but rather as situated in the wall of the chest 3.5 cm. below the midclavicular area. It was *attached to* and elevated the skin.

Budd's sections are interesting. In one there is a small focus, well outside the main tumor, where a small sweat gland appears with a dense

18. Budd, J. W., and Breslin, F. J.: Am. J. Cancer **31**:207, 1937.

hyaline stroma. This minute area resembles a cylindromatous sweat gland tumor though it is scarcely large enough to be a tumor at all. Nevertheless this finding gives a hint as to the nature of the main tumor and the basis for its general resemblance to mammary cancer. It also affords a reason, based on the known behavior of sweat gland tumors, why the tumor grew so slowly. There is evidence that the main tumor had long possessed a dense hyaline connective tissue capsule and that extensive hyaline alterations in the stroma had been present. Also the surrounding vessels are markedly thickened. In one section there is evidence of widely dilated lymph (?) spaces with some blood, or possibly of blood spaces, suggesting the stasis seen in other lesions where ossification or calcification has supervened. The question in our minds is whether the bone is being formed or being destroyed, and it appears to us that destruction is obvious, whereas new formation of bone is at least doubtful. We are not convinced that osteoblasts are present, and, on the other hand, clasmatocytes are numerous. The latter are found also in the midst of areas of obvious cancer, and the only known reason for their being present in such areas is that these zones previously contained bone or calcified material which had disappeared under the cancerous invasion.

Differences of interpretation are possible as to the relation of the epithelial tumor cells to the bone. It is certain that in cross section many tumor cells appear to be surrounded by hyaline matrix or bone, but, on the other hand, it is also clear that malignant tumor cells are to be found invading bone from without, in penetrating strands, and that cells apparently surrounded by bone may be found in rows of two or three like an epithelial tumor. Where bone is farthest removed from surrounding cancer, we do not find obvious cancer cells in the bone but only bone cells or empty spaces. In our opinion, the malignant cells found within the bone are cells of an epithelial tumor; the spindle cells represent metaplastic epithelial cells, and we therefore agree with Ewing that the tumor is purely epithelial. We believe that the resemblance to osteogenic sarcoma is striking but that on more minute analysis it falls down and that we are dealing with an old calcified, ossified epithelioma, probably of sweat gland origin, in which cancer has developed, sweat gland cancer often having a close resemblance to classic mammary cancer for obvious reasons, and that the resemblance to osteogenic sarcoma is the result of rapid invasion and dissolution of preformed stromal bone by cancer. Thus the process differs from what is usually considered a malignant mixed tumor. We apologize for offering an unsolicited fourth opinion on this interesting tumor. After we had reached our opinion, there appeared in the literature a description of a similar tumor by Tudhope,[19] concerning which he reached a conclusion nearly similar

19. Tudhope, G. R.: J. Path. & Bact. **48**:499, 1939.

to ours, the difference being that his tumor was originally definitely mammary fibroadenoma.

A resemblance to osteogenic sarcoma is seen in a curious, exceedingly vascular mammary cancer in which the events leading to the histologic similarity between the carcinoma and osteogenic sarcoma are at least partially traceable.

The tissue surrounding the tumor is made up of hypertrophic mammary lobules with their branches widely separated by edematous connective tissue infiltrated with lymphocytes. The mammary fat tissue is unusually vascular and almost lipomatoid. In certain of the lobules small invaginations of duct epithelium have developed. Both in these invaginations and in the lumens of the glands are prominent calcific psammoma bodies. The indications are that the deposition of calcium occurs in areas of intraductal hemorrhage. A source of calcium for subsequent events is thus present.

The tumor itself is obviously a low grade comedo carcinoma where it can be recognized as carcinoma at all. Now evidently a circulatory accident has occurred which has resulted in communication between the ducts, lined by comedo carcinoma, and vessels, with conversion of part of the tumor into a markedly telangiectatic structure. It is possible that in the telangiectatic area a calcium surcharge is present. Indications of an excess of calcium have already been noted. At any rate, large numbers of epulis-like giant cells are present in the area; the resemblance of the lining cells to comedo carcinoma is so altered that the separated cells cannot readily be identified as epithelial; an effusion of blood together with cells occurs into the loose connective tissue, where resemblance to epithelium is further lost. Tumor giant cells, epulis-like giant cells, telangiectasia and loose tumor cells, no longer recognizable as epithelial in origin, complete the resemblance to telangiectatic osteogenic sarcoma.

In another tumor of similar nature the epulis giant cells are so numerous that wide areas duplicate the structure of benign giant cell tumor of bone.

In our cartilage-containing tumors of the human breast, tumors resembling the common cancer of the canine breast, we find no satisfactory explanation for the appearance of the cartilage. It seems to be associated with a mucoid edema of the spindle cell portion of a mixed "carcinosarcoma," obviously in some instances a development in a preexisting fibroadenoma. The edematous connective tissue is rather vascular, being well equipped with capillaries having only an endothelial wall. The evidence of stasis previously described is not found in these edematous areas, and occlusive hyalinization of vessels is not seen. The static type of circulation may appear later, after the formation of rather adult-looking cartilage, but then it forms a sort of cavernous plexus about

the cartilaginous areas. The evidence in our material is insufficient to indicate that the circulatory changes are causal, and the reason for the characteristic cartilaginous development in these tumors is totally obscure. To call them mixed tumors or to invoke a teratoid origin evades the question.

It is evident from another mammary tumor—a pure epithelial "carcinosarcoma"—that the static type of circulation with hemorrhage is not alone sufficient to produce calcification or ossification in the mammary tumor. The lesion is a circumscribed solid adenocarcinoma made up of plexiform sheets of closely packed small polyhedral cells. In places the closely packed areas of epithelial cells give way to progressively looser areas where the picture changes from one in which the resemblance to condensing mesenchyme is striking to one in which the epithelial character of the cells is lost through their becoming mingled diffusely with mononuclear phagocytes, plasma cells, loose elements of areolar tissue, giant cells and blood. In these loose areas there is hemorrhage; the vessels are widely dilated and have only a single endothelial layer, and one gets the impression of marked stasis. However, no hyaline tissue is found, nor does one find evidence of calcium. Apparently, despite the presence of a circulatory picture found in other instances in which ossification has occurred, the connective tissue fails to provide through preliminary hyalinization an ossifiable medium.

The relation of heterotopic bone formation to blood vessels is seen in the evolution of a curious tumor [20] of the lower extremity. A series of biopsy specimens show the progress of the lesion. The process begins in multiple small, lobule-like capillary angiomas (fig. 1). These lie in fat tissue, but the fat lobules appear rather fibrosed, and in the interstices between the capillaries the active cells appear to be spindle cells of the connective tissue rather than fat cells. In each of the smallest lobules is a central vessel of the caliber of a small arteriole. This branches in all directions toward the periphery of the circumscribed lobule, the terminal branches being capillary in type. The larger vessels show a poorly developed muscularis, the cells of which are poorly differentiated from fusiform or branched connective tissue cells occupying the intervening loose areolar tissue; in cross section the cells of the muscularis of certain of the vessels suggest the myoid cells of the glomus. The intervening areolar tissue is somewhat edematous and contains a fine fibrillar deposit. Not all of the vascular lobules show the capillary structure. Some seem entirely made up of thick wall arterioles, and the lesion resembles more a varix.

As in previous examples of bone formation, the first event seems to be extensive progressive hyalinization of the capillary wall. This proceeds until the layer of hyaline material exceeds the diameter of the vessel in

20. Dr. Howard Permar gave us permission to use this case.

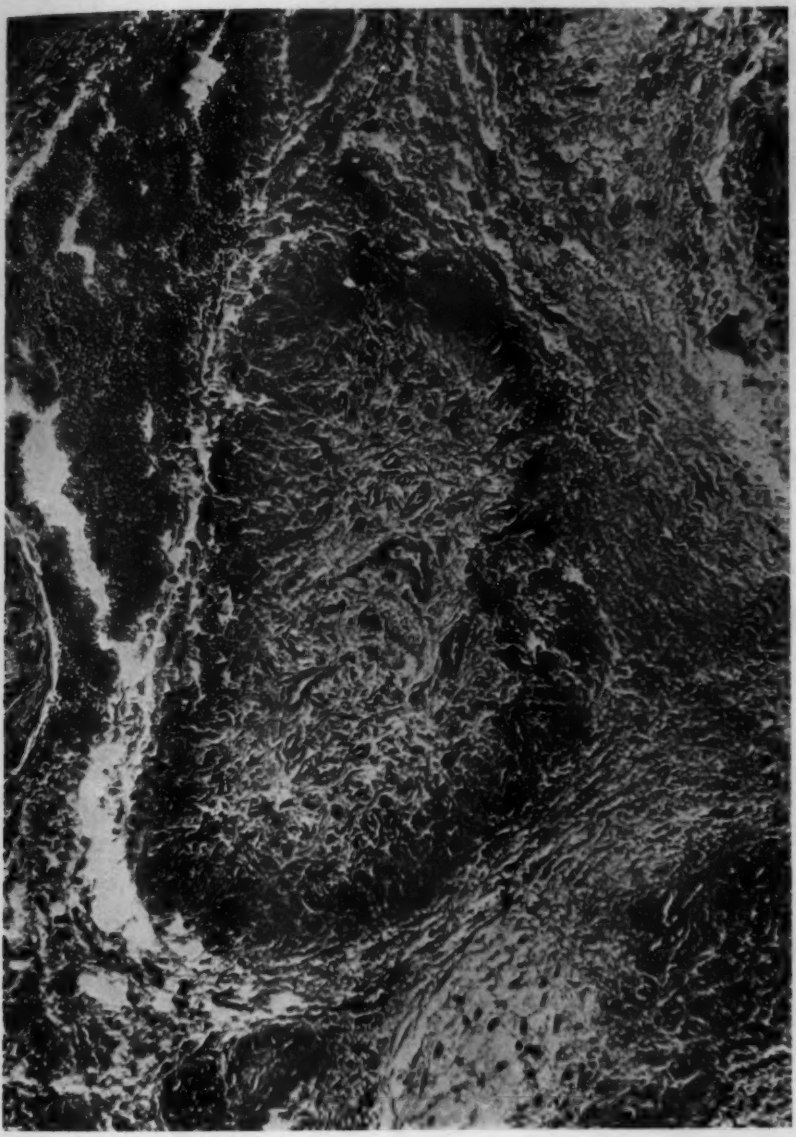

Fig. 1.—One of a group of lobular angiomas, prior to the development of perivascular hyalinization.

thickness or even until obliteration of the capillary has occurred (fig. 2). The entire capillary bed may disappear, and the residual picture may be that of a cavernous angioma surrounded by thick hyaline material. It is in these hyaline areas that calcification and poor ossification occur.

The neoplastic process in its malignant phase (fig. 3) develops in the individual hyalinized or calcified or ossified vascular lobules as a malignant multicentric tumor. We cannot be certain of the exact cells of origin, i. e., whether muscle or connective tissue, but they are clearly not cells of the vascular endothelium. We believe them to be connective tissue cells. The structure of the tumor depends largely on the character

Fig. 2.—Later stage showing perivascular hyalinization and partial occlusion of vessels. Perivascular tumor cells are present.

of the process in the vascular lobule at the time the malignant phase begins. In the presence of extreme hyalinization the appearance suggests that of an osteoid osteosarcoma. With marked calcification or ossification the resemblance is to osteogenic sarcoma (fig. 4), although nowhere is there clearcut evidence that malignant tumor cells are forming bone; they rather occupy interstices between hyaline bundles and calcified matrix, which they invade and in which they develop. The resemblance to bone sarcoma is intensified by the presence of numerous tumor giant cells together with giant cells of the epulis type both in the hyaline and in the calcified areas.

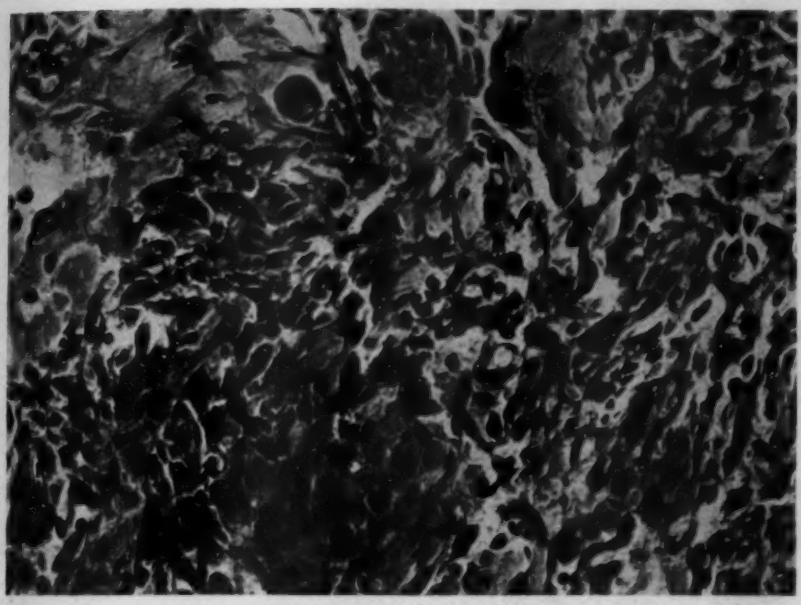

Fig. 3.—The same tumor, more atypical and malignant looking, and showing resemblances to osteoid sarcoma.

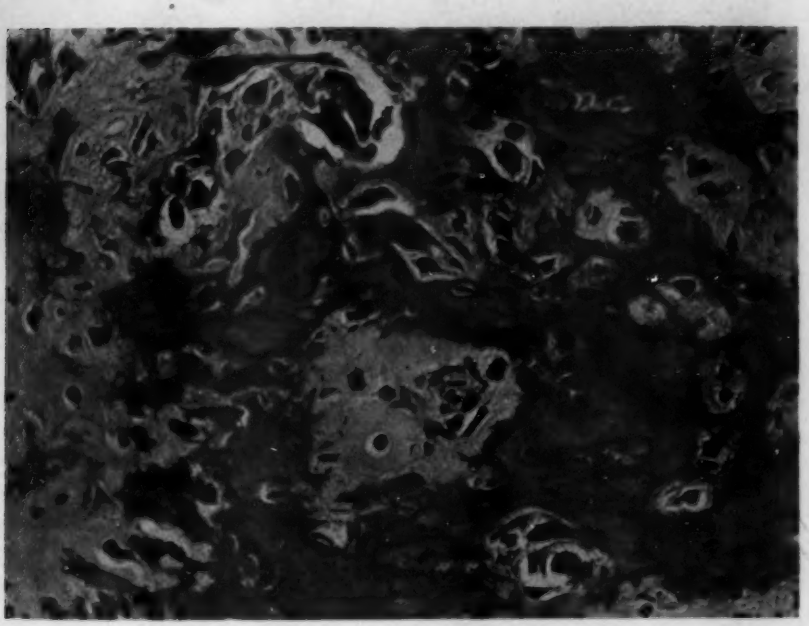

Fig. 4.—One of many areas of bone formation, where the lesion resembles osteogenic sarcoma.

Here the malignant tumor is something which has been added to an originally benign angioma which underwent degenerative changes so altering the circulation that calcification and ossification occurred. The malignant tumor developed in multiple lobules under an unknown stimulus. Our study has clarified conditions responsible for the histologic character of the malignant tumor but not its etiologic nature. No source of calcium surcharge is known; the tumor was in close proximity to the tibia, which may have been injured in early operations.

This relation of vascularity to calcification is seen in another interesting tumor in the Memorial Hospital collection. The tumor is a uterine angiomyoma, whose original structure probably corresponded to that

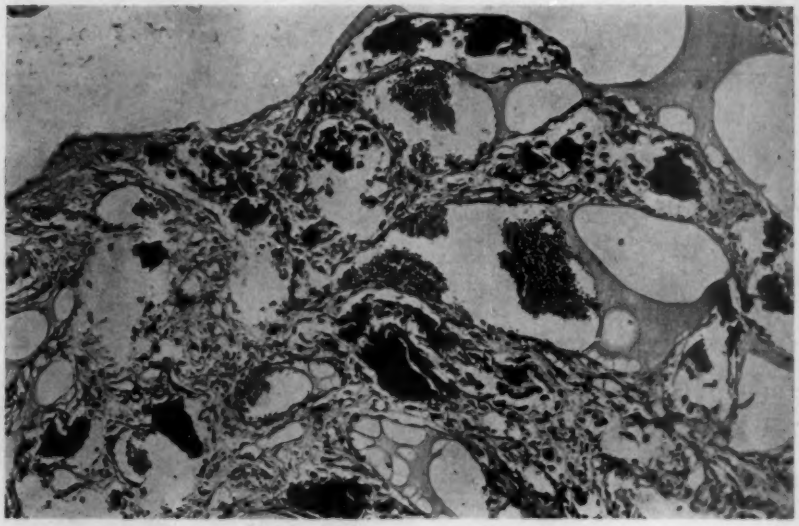

Fig. 5.—Areas of telangiectatic cavernous vessels found between myomatous bundles; calcification, now showing signs of resorption, in the interstices; cells resembling bone cells in the calcific material.

described by Pusch [21] as "ramifying angiomyoma of the uterus." In this tumor the myomatous elements are arranged about cavernous blood vessels which in some areas constitute essentially a cavernous angioma surrounded by a connective tissue sheath inside and a myomatous sheath outside. The major vessels outside the tumor are greatly thickened. Bone is not laid down where the vessels are thick walled, sclerotic and hyalinized, but rather it seems to appear where they are thin walled and aneurysmal and where the picture suggests stasis (fig. 5). There is no evidence that the muscle cell is concerned. The calcification occurs in the connective tissue, and the connective tissue cell becomes

21. Pusch, L. C.: Am. J. Obst. & Gynec. **24**:907, 1932.

incorporated as a bone cell. No cells resembling osteoblasts are seen. In some areas, after bone formation has occurred there is evidence of vascular occlusion with infarction, death of bone and reaccumulation of lime salts in the hyalinized connective tissue.

Although the benign tumor was clearly a perivascular uterine myoma, the malignant tumor which developed in the areas of calcification and ossification appears to be a connective tissue tumor. We first accepted the specimen as one of osseous metaplasia of myomatous tissue or osseous metaplasia in a myosarcoma but after much study were forced to abandon that concept. The malignant change begins in the connective tissue in multiple areas. It can be followed in separate perivascular

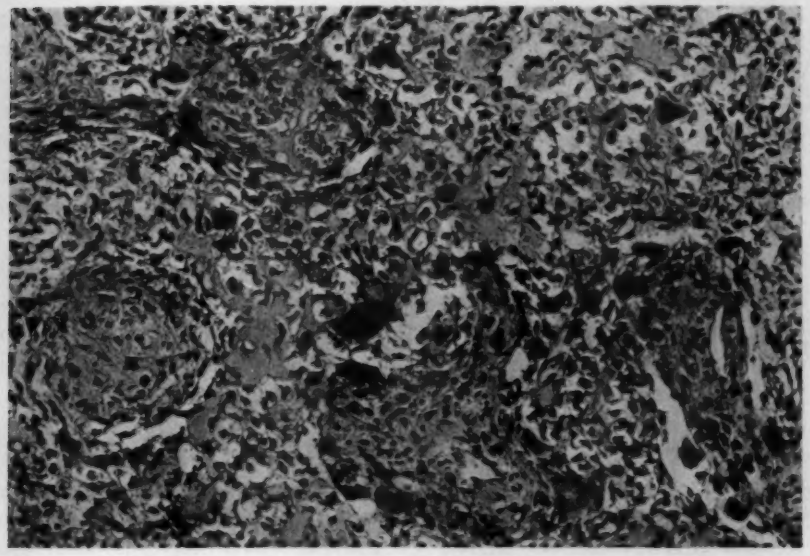

Fig. 6.—Actively malignant area of the same tumor showing a structure indistinguishable from osteogenic sarcoma.

units surrounded by muscle, which later it invades. Thus the tumor is a connective tissue sarcoma invading preformed membrane bone and calcified and noncalcified hyaline tissue, and its resemblance to osteosarcoma is a consequence of this fact (fig. 6). As usual the similarity is intensified by the mingling of epulis-like giant cells with true tumor giant cells and the occurrence of masses of hyaline tissue resembling osteoid hyalin, which material, however, we interpret as originally the result of degenerative perivascular hyalinization rather than as a new product formed under the influence of the malignant tumor cell. In fact, the appearance of the tumor cells suggests cells too active in the direction of growth to be concerned in the laying down of collagen. The reason

for the development of a malignant connective tissue, pseudo-osteogenic sarcoma in an angiomyomatous uterus is unknown. The conditions believed responsible for the osteogenesis are traced in part, but these naturally afford no basis for understanding the development of the sarcoma itself.

Barringer and Woodard [22] recently described a peculiar prostatic tumor in the Memorial Hospital series. The lesion presented the combined features of typical prostatic cancer and a calcifying chondromatous tumor surrounded by what at first appeared to be spindle cell sarcoma. Naturally the latter bore certain resemblances to periosteal sarcoma. The blood serum of this patient yielded three to four times the normal level of alkaline phosphatase, although the patient showed neither clinical nor roentgenographic evidence of bone metastases from prostatic cancer. The acid phosphatase showed no increase, and it was therefore suggested that the picture in the prostatic tumor—the calcifying chondromatous portion—was due to some alteration in the prostatic phosphatase to a type capable of inducing calcification. However, we do not know of the relation of phosphatase to the development of cartilage, and although an altered phosphatase may be invoked to explain calcification, it scarcely explains the preliminary development of cartilage.

The sections are interesting. The cancer itself is not unusual. It is a rather malignant-looking adenocarcinoma, solid and alveolar. The cells contain rather more lipoid material than do those of the average prostatic cancer. In the midst of the obvious carcinoma is a large mass of amorphous calcific material and about the latter is a zone of fairly normal-appearing cartilage. The cartilage itself is in turn surrounded by a thick zone of dense fibrous tissue, in places almost acellular, in others containing shrunken nuclei like those of quiescent connective tissue cells, and in still others, large malignant tumor cells. The temptation is to regard the lesion as a metaplastic osteogenic sarcoma, and this indeed was our first interpretation.

However, the calcification is occurring in areas where there are no cells which are possibly interpreted as of epithelial origin. The cartilage seems to develop following the appearance of a mucoid edematous state in the dense connective tissue, although we have not been able to assure ourselves that the transition between connective tissue cells and cartilage cells is a clear one. The cartilage cells do not appear definitely neoplastic, although in some areas one might be justified in calling them chondromatous. Confusion is the result of invasion of preformed cartilage by epithelial tumor cells. The latter occur as single elements and as narrow strands of three or four cells. They show the cohesion of

22. Barringer, B. S., and Woodard, H. Q.: Tr. Am. A. Genito-Urin. Surgeons **31**:363, 1938.

epithelial cells and the acidophilic cytoplasm of those of the prostatic carcinoma. Hence the appearance of metaplastic osteogenic sarcoma is the result of confusion of two distinct processes. We are unable to offer any explanation for cartilage in the prostate. The alkaline phosphatase is probably referable to the local calcifying lesion, since no sign of metastases in bones have as yet appeared, fifteen months after operation.

In another prostatic carcinoma conditions which might well have led eventually to the appearance of pseudo-osteogenic sarcoma are apparent. The principal lesion is typical prostatic cancer. However, in numerous areas there are peculiar osteoid changes in the stroma and in the surrounding bladder muscle and connective tissues. These consist in part of hyalinization of muscle and fibrous tissue and in part in extreme perivascular, mainly pericapillary hyaline deposits. In places capillaries are totally obliterated, and the residual endothelial cells are so reduced through atrophy and disappearance that they resemble isolated future bone cells of osteoid trabeculae. Calcification is beginning in certain of these osteoid trabeculae, and in many areas one finds nothing but clusters of cancer cells occupying the interstices of the latter. From a picture of this sort it is but a short step to an appearance which could be readily confused with metaplastic osteogenesis of epithelial origin. No determinations of phosphatase in this tumor are available.

True heterotopic osteogenic sarcoma is found in a large malignant, metastasizing tumor apparently primary in the pericardium at the base.

The patient was a youth of 19 years. His initial symptom was described as an attack of pleurisy six months prior to admission. He stated that he had remained well thereafter until four days prior to admission, when he had a series of hemoptyses followed by cough, dyspnea and swelling of the face, neck, right arm and right leg. Clinical and roentgenographic examinations revealed what was thought to be a bulky mediastinal tumor. Metastases were demonstrable in the soft tissues of the left thigh, and a neurologic consultation furnished evidence of cerebral metastases. Death occurred two months later.

Autopsy revealed what was interpreted as a primary osteogenic sarcoma of the pericardium with metastases to the brain, pancreas, kidney, lungs and soft tissues of the left iliac fossa and left thigh. Pulmonary edema, pleurisy with effusion, thrombosis of the left common and internal jugular veins and massive hemopericardium were terminal events.

In our opinion this tumor is not a teratoid osteogenic sarcoma but one arising as a metaplasia in fibrous tissue. It surrounds the cardiac base and appears to arise from the pericardium. At least the latter can be traced into the tumor as a thin lamina for a short distance, whereupon it becomes lost. Other regional anatomic structures are identified as distinct from the tumor and viscera; the heart, trachea, esophagus and lungs are merely pushed aside. Evidence for preexisting inflam-

mation of thoracic serous membranes was found in the presence of numerous old fibrous pleural adhesions, which required separation by sharp dissection.

The tumor did not begin, in all probability, as an osteogenic sarcoma. The active portion is pure fibrosarcoma. The bone-forming areas suggest parts left behind in the course of active growth. They show the structure of osteoid bone developing in hyalinized connective tissue and occasionally well formed dense bone, whose architecture nevertheless reveals its origin in the osteoid trabeculae. Tumor cells are incorporated as bone cells and even tend toward vacuolation and the formation of what resembles marrow fat. Sections stained specifically for fat are not available. In the osteoid and osseous areas the blood supply consists of thin-walled, widely dilated vessels. In the most actively growing periphery of the tumor no such type of circulation is seen. It is not possible, however, to tell with certainty whether the circulatory type favoring stasis precedes, i. e., whether it is a factor in, the osteogenesis, or follows the latter, but we believe it to be a factor, for in certain of the cellular non-bone-forming areas the telangiectatic features are found developing. When the preosseous hyaline material can be definitely localized in reference to the markedly dilated vessel, it is seen to be definitely away from it, i. e., in the least well nourished area and separated from the vessel by spindle cell tumor. Completion of the osteoid change finds the dilated vessel and osteoid tissue in much closer approximation. Mucoid-looking edema of the osteoid tissue precedes true ossification. Osteogenesis is absent in the metastases, which show the structure of pure spindle cell sarcoma. Since we have no reason to assume different cell potencies in the metastases, the assumption that osteogenesis in the primary tumor is the result of specific local conditions rather than of specific cell potencies is probably justified.

SUMMARY

A number of tumors of soft tissue having an epithelial origin but bearing some resemblance to osteogenic sarcoma are described, and an effort is made to discover morphologic changes of importance which lead to this resemblance. It may be concluded that the most important alterations leading to the assumption of the structure of osteogenic sarcoma are the laying down of dense hyaline tissue, resulting probably in ischemia, and the development of a cavernous telangiectatic type of circulation favoring stasis and consequent probable anoxemia. These features fail to explain the structure of cartilage-containing mixed tumors, and some other mechanism, as yet undetected, must be invoked.

BLOOD PRESSURE IN EXPERIMENTAL NEPHRITIS PRODUCED BY INJECTION OF NEPHROTOXIC SERUM

CALVIN F. KAY, M.D.*

BALTIMORE

In 1933 Masugi [1] demonstrated that when rats were given injections of serum from rabbits immunized against rat kidney acute glomerulonephritis resulted. Previously nephritis had been produced repeatedly with anti-kidney serum by Lindemann,[2] Pearce,[3] Woltmann [4] Wilson and Oliver [5] and others. Masugi, however, in reviving and improving on this almost forgotten method, made a much more thorough study. He was the first to show incontestably the close clinical and pathologic resemblance of this disease to human glomerulonephritis. In a second series of experiments, in 1934, he [6] produced a similar form of nephritis in rabbits by injections of anti-rabbit-kidney duck serum. His work has been amply confirmed by many investigators, including Hemprich,[7] Weiss,[8] Smadel,[9] Arnott, Kellar and Matthews,[10] Ehrich,[11] Korányi and Hámori,[12] Tsuji [13] and Ogawa and Sato.[14]

With the development, for the first time, of experimental nephritis closely simulating the human disease, many new avenues of investigation were opened. In the course of a study of experimental nephritis produced by nephrotoxic serum I have been interested in observing the changes in blood pressure that accompany the disease in rabbits.

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10. Arnott, W. M.; Kellar, R. J., and Matthew, G. D.: Edinburgh M. J. **43**: 233, 1936.
11. Ehrich, W. E.: Proc. Soc. Exper. Biol. & Med. **35**:576, 1937.
12. Korányi, A., and Hámori, A.: Ztschr. f. klin. Med. **130**:774, 1936.
13. Tsuji, S.: Beitr. z. path. Anat. u. z. allg. Path. **98**:424, 1937.
14. Ogawa, S., and Sato, Y.: Tr. Soc. path. jap. **28**:212, 1938.

Masugi [6] determined the systolic blood pressure in his second series of experiments, in which rabbits became nephritic when given injections of anti-rabbit-kidney duck serum. He used the capsule method of von Recklinghausen [15] on the denervated ear. The blood pressure in normal rabbits ranged from 60 to 80 mm. of mercury. Hypertension was reported in 5 of 6 animals. Maximum rises of 38 and 17 mm. were observed in the 2 rabbits with the most intense nephritis. In 3 animals with less marked nephritis elevations of from 9 to 27 mm. occurred. In 1 rabbit the disease was very mild, and no rise of blood pressure was observed. When hypertension occurred, it always preceded the onset of albuminuria. In 1 rabbit the pressure returned to normal before the appearance of the albumin in the urine and did not rise subsequently. The longest duration of hypertension was fifty-seven days.

Arnott, Kellar and Matthews [16] determined systolic blood pressure in nephritic rabbits by the carotid loop method of van Leersum.[17] Certain modifications had been adopted as reported in a previous paper by Arnott and Kellar.[18] Thirty animals were studied. Charts and protocols are shown for 5 representative normal animals given injections of five different nephrotoxic serums. Hypertension was invariably observed. The degree depended more on the particular lot of serum used than on the degree of histologic change in the kidney. In some animals the blood pressure rose within twenty-four hours after the injection of nephrotoxic serum, but in others the elevation did not occur until ten days had elapsed, thus showing an inconstant relation to the appearance of albumin in the urine. Hypertension lasted for from two weeks to "a permanent elevation in pressure." From normal levels of 80 to 100 mm. of mercury, rises of from 20 to 40 mm. were reported.

In a second series of experiments 7 rabbits were subjected to renal denervation and were later give serum known to be potent. Clinically and histologically, the ensuing nephritis was not significantly different from that observed in the previous experiments, but elevation of blood pressure did not occur. In a third group, of 5 animals, renal denervation terminated already established hypertension. They concluded that their results "strengthen the contention that the hypertension of acute diffuse renal disease depends for its occurrence upon the integrity of the renal nerve supply."

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15. von Recklinghausen, H.: Arch. f. exper. Path. u. Pharmakol. **55**:375, 1906.

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17. van Leersum, E. C.: Arch. f. d. ges. Physiol. **142**:377, 1911.

18. Arnott, W. M., and Kellar, R. J.: J. Path. & Bact. **42**:141, 1936

A somewhat similar study has been reported by Hámori and Korányi,[19] who came to opposite conclusions. Blood pressure was determined by use of an ear capsule as described by Grant and Rothschild.[20] In the first experiments they made readings on 3 nephritic animals. In the control period the average pressure ranged from 45 to 60 mm. of mercury. There was a rise of from 7 to 10 mm. during the incubation period, between the injection of serum and the appearance of albumin in the urine. In 2 rabbits a drop toward normal was followed by a secondary rise of 15 and 40 mm. of mercury, respectively, following the onset of the nephritis. In the third animal no secondary rise occurred, although the nephritis was severe.

In a second series of experiments [19a] 4 animals were studied. Two were subjected to bilateral and a third to unilateral renal denervation before nephrotoxic serum was given. The dosage was not uniform. The changes in blood pressure were essentially the same as in those animals in which the renal nerve supply had been left intact. They concluded that hypertension is essentially independent of renal innervation, although the levels attained may be somewhat lower when the renal nerve supply has been destroyed.

Smadel and Farr [21] induced nephritis in rats by the injection of anti-rat-kidney rabbit serum. An ear capsule as described by Moberg [22] was used in determining the blood pressure. Hypertension was found to develop in 2 animals in the eighth month of chronic progressive nephritis. From a normal range of from 50 to 60 mm. of mercury, pressures in these animals rose to approximately 100. No elevation of blood pressure was found to occur in the acute phase of the disease.

METHODS

Determination of Blood Pressure.—A capsule similar to that described by Grant and Rothschild [20] was prepared. Condom rubber was used, loosely applied, in place of the gold beater's skin originally recommended.

Satisfactory readings could not be obtained when the central artery of the ear was constricted. In order to obtain constant vasodilatation the rabbit was placed on a warm electric pad covered with several layers of muslin. It was held by a towel which was applied firmly but not tightly. In this position the rabbit was usually quiet and comfortable, and the arteries became fully dilated.

The capsule was then applied to the ear. Elevation of pressure within the capsule repelled the flexible membrane, compressing the central artery of the ear until it could no longer be seen to pulsate. The pressure was then allowed to fall

19. (*a*) Hámori, A., and Korányi, A.: Ztschr. f. klin. Med. **133**:722, 1938. (*b*) Korányi and Hámori.[12]

20. Grant, R. T., and Rothschild, P.: J. Physiol. **81**:265, 1934.

21. Smadel, J. E., and Farr, L. E.: J. Exper. Med. **65**:527, 1937.

22. Moberg, W.: Skandinav. Arch. f. d. ges. Physiol. **69**:218, 1934.

gradually. When a distinctly visible pulsation abruptly reappeared, the pressure measured in millimeters of mercury constituted a reading. An interval of three to five minutes was then allowed to elapse, after which the capsule was reapplied to the ear and another reading obtained. The average of three or more consecutive *readings* varying less than 3 mm. of mercury constituted one *measurement*. Measurements were made at intervals of twenty-four hours or longer on each rabbit.

The foregoing method of determining blood pressure was adopted for several reasons. In principle it is the same as the method employed by Landis, Montgomery and Sparkman [23] and by Pickering and Prinzmetal.[24] Landis and associates,[23] employing a graphic method, found that an ear capsule gave systolic blood pressure readings that were almost identical with those simultaneously obtained in the opposite ear by the method of Hamilton, Brewer and Brotman,[25] both in the normal rabbit and in those that were made hypertensive by abdominal constriction, asphyxia or an injection of epinephrine. The systolic pressure in the central artery of the ear was found to be 12 to 23 mm. of mercury lower than that in the femoral artery. Pickering and Prinzmetal,[24] as well as Landis and associates,[23] found the ear capsule method adequate to detect rises in pressure caused by injection of pressor extracts obtained from the kidney.

Figure 1 shows the response of the systolic blood pressure to intravenous injections of a renal pressor extract, prepared by a method similar to that of Landis and associates, as revealed by the blood pressure method employed in this investigation.

Constant dilatation of the central artery of the ear is essential for consistent measurements of blood pressure. In my hands the requisite dilatation has been obtained best by warming the animal. A quiet, dark environment, recommended by Korányi and Hámori,[12] when used alone was not found to be adequate. In the few animals in which sympathetic denervation of the ears was performed, as recommended by some,[26] continuous full dilatation of the central artery was not obtained.[27] A water-warmed box [23] for heating the rabbits was discarded for an electric heating pad because the use of the latter was simpler, and the animals less frequently became restless and uncomfortable.

The van Leersum method [17] in my hands did not give consistent results from day to day. Moreover, the method is open to criticism, for

23. Landis, E. M.; Montgomery, H., and Sparkman, D.: J. Clin. Investigation **17**:189, 1938.

24. Pickering, G. W., and Prinzmetal, M.: Clin. Sc. **3**:211, 1938.

25. Hamilton, W. F.; Brewer, G., and Brotman, I.: Am. J. Physiol. **107**:427, 1934.

26. Masugi.[6] Hámori and Korányi.[19a]

27. Grant, R. T.: Clin. Sc. **2**:1, 1935.

it is well known that occlusion of the carotid artery may bring about a rise in systemic blood pressure through the activity of the carotid sinus mechanism.[28]

Production of Nephritis in the Rabbits.—Nephrotoxic duck serum was prepared by a modification of the method of Masugi.[6] Ducks were immunized by repeated intraperitoneal injections of a saline suspension of perfused, crushed rabbit kidney. Streptococcus toxin was given concomitantly to most of the ducks as a nonspecific antigen. Details of these procedures will be described elsewhere.

Nephrotoxic duck serum was injected intravenously into 19 rabbits. In each animal after an incubation period of from five to ten days there was an abrupt onset of clinical nephritis—manifested by the presence in the urine of large amounts of albumin, many casts and fairly large numbers of leukocytes and red blood cells. Specimens of urine obtained a day or two immediately after injection frequently

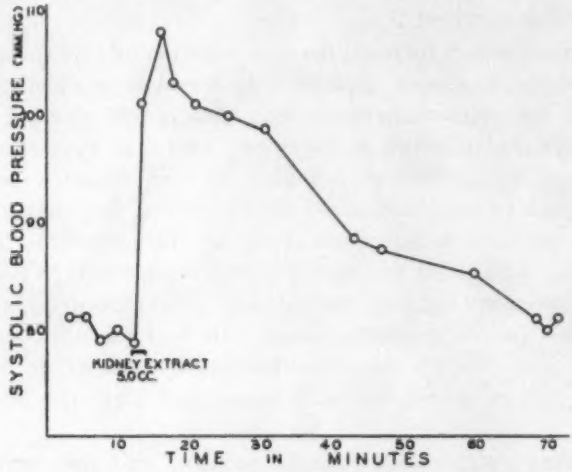

Fig. 1.—Systolic blood pressure response to 5 cc. of kidney extract (prepared by a modification of the method described by Landis and associates [23]) as indicated by the method of determining blood pressure used in this study.

contained small amounts of albumin, but this always disappeared before the onset of true nephritis.

The clinical and pathologic characteristics of the nephritis in any particular animal depended on the potency and dose of the serum which had been injected. In mild nephritis (occurring in 11 of the 19 rabbits) the health of the animal seemed generally unimpaired, and the blood nonprotein nitrogen did not rise above the normal range of from 25 to 50 mg. per hundred cubic centimeters. The urinary abnormalities gradually subsided to disappear entirely (except perhaps for a trace

28. Verney, E. B., and Vogt, M.: Quart. J. Exper. Physiol. **28**:253, 1938. Grant.[27]

of albumin in concentrated specimens of urine) in from fifty to one hundred days. The active period was considered to last from the onset of nephritis until the urine contained less than 250 mg. of albumin per hundred cubic centimeters. Thereafter the animal was in the recovery period.

A rise in blood nonprotein nitrogen (at least two determinations of 60 mg. or more per hundred cubic centimeters) was used as the criterion to differentiate severe nephritis (occurring in 8 of the 19 rabbits) from mild nephritis. Many of the rabbits with severe nephritis appeared ill, ate poorly and lost weight. One of the animals died in uremia sixteen days after the injection of the nephrotoxic serum. The urinary abnormalities were qualitatively the same in severe as in mild nephritis, but they were more marked in degree and longer in duration in the 7 animals which survived.

The nephritis which followed the first injection of nephrotoxic serum has been designated primary nephritis. In 8 rabbits in which all clinical evidences of the primary nephritis had disappeared (except in some instances a trace of albumin in the urine) nephritis was reprecipitated by again injecting nephrotoxic serum. In each instance, before the second injection of nephrotoxic serum the animal was desensitized by giving eight intravenous injections of normal duck serum in increasing doses, starting with 0.001 cc., during a two day period. This desensitization was necessary to avoid anaphylaxis. The reprecipitated nephritis differed little from the primary disease. In 3 of the 8 animals it was of the mild type. Severe nephritis developed in the other 5 animals, 3 of which died in uremia ten to nineteen days after the injection of nephrotoxin.

The clinical characteristics of the primary and the reprecipitated severe nephritis are illustrated in the case of rabbit 117:

This normal 1,900 Gm. rabbit received normal duck serum in doses of 0.018 cc. and 0.18 cc., respectively, on consecutive days in divided doses.[29] The following day 1.0 cc. of nephrotoxic duck serum, lot X, was injected. Albumin first appeared in the urine on the seventh day and became massive the following day. The urinary sediment contained many hyaline casts and leukocytes and a few white cell casts, red cell casts and red blood cells. Nonprotein nitrogen rose from a control level of 37 mg. to 110 mg. per hundred cubic centimeters on the eleventh day and remained elevated for about four weeks. Albumin continued to be abundant in the urine for about two more weeks, but the casts and cells diminished in numbers. Thereafter urinary abnormalities gradually diminished and by the hundredth day the only remaining clinical evidence of the nephritis was a faint trace of albumin in concentrated urine specimens.

29. Although desensitization was actually necessary only in animals which had previously received injections, the administration of nephrotoxin was almost always preceded by a standard two day desensitization period so that the dosages in original and reinjection nephritis would be strictly comparable.

The animal was then desensitized and given 1.0 cc. of nephrotoxin, lot X, exactly as before. No signs of anaphylaxis were observed. The ensuing nephritis was severe and characteristic of the reprecipitated disease. It differed little from the primary nephritis in the same animal. The incubation period was shorter, and the rise in nonprotein nitrogen was not quite as great. Urinary abnormalities and the course of the disease were essentially the same as had previously been observed. Blood pressure measurements in this animal are illustrated in figure 2.

Seven rabbits which had recovered from an attack of primary nephritis were desensitized in the usual manner and given injections of normal duck serum. Nephritis developed in 3 of these animals. One (rabbit 128) showed mild nephritis and was killed sixteen days after injection. One (rabbit 82) died in uremia at the end of thirty-six days.

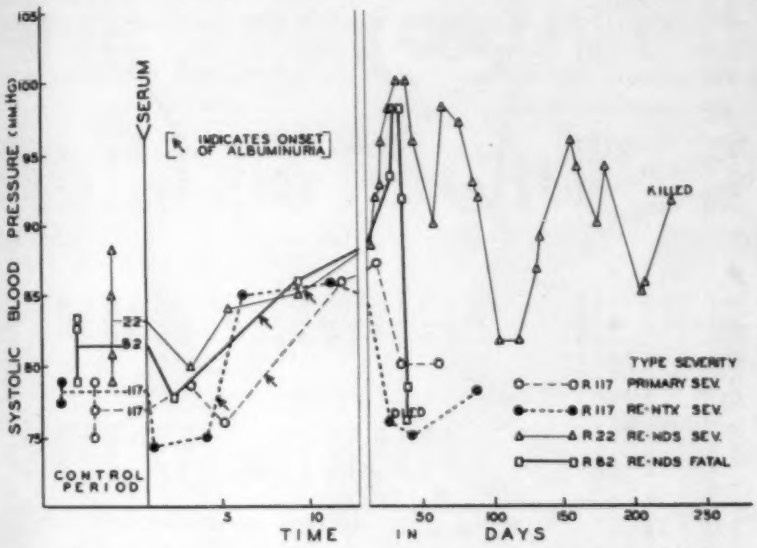

Fig. 2.—Systolic blood pressure in millimeters of mercury in 3 rabbits through four courses of nephritis. Each measurement is represented by a symbol (circle, triangle or square) and was derived from several readings. The abbreviations are as follows: *R*, rabbit; *RE-NTX*, reprecipitated nephritis, from injection of nephrotoxic duck serum; *RE-NDS*, reprecipitated nephritis, from injection of normal duck serum; *SEV.*, severe.

One (rabbit 22) was killed two hundred and twenty-nine days after receiving the injection, while the clinical nephritis was still in the active stage. The urinary findings for these animals were essentially the same for the other nephritic animals. Red blood cells and red cell casts were not more numerous. The pathologic process seemed to be the same, although the histologic appearance of the kidneys was altered by the duration of the disease. In rabbit 22 the nonprotein nitrogen was consistently greater than 100 mg. per hundred cubic centimeters for

TABLE 1.—*Mean Systolic Blood Pressure of Each Rabbit by Type of Nephritis, Severity of Nephritis and Period of Disease*

	Primary Nephritis					Reprecipitated Nephritis				
		Mean Systolic Blood Pressure (Mm. of Mercury) with Number of Measurements* (in Parenthesis)					Mean Systolic Blood Pressure (Mm. of Mercury) with Number of Measurements* (in Parenthesis)			
Rabbit	Severity of Nephritis	Control Period	Incubation Period	Active Period	Recovery Period	Severity of Nephritis	Control Period	Incubation Period	Active Period	Recovery Period
17	Mild	.....	.....	.....	.....	Severe	77.3 (3)	66.0 (2)	81.8 (5)	Died
21	Severe	.....	.....	.....	.....	Severe	80.3 (3)	74.0 (1)	81.5 (4)	Died
22	Severe	.....	.....	.....	.....	Severe†	83.3 (4)	83.0 (3)	92.1 (24)	
121	Mild	80.0 (3)	.....	77.5 (2)	77.5 (2)	Mild	76.0 (2)	71.0 (2)	77.0 (4)	77.8 (4)
95	Mild	81.7 (3)	78.0 (2)	80.5 (4)	83.5 (2)					
230	Mild	76.0 (3)	74.5 (4)	75.5 (2)	78.3 (3)					
92	Mild	84.0 (3)	.....	85.0 (3)	83.0 (2)	Mild	84.5 (2)	85.0 (1)	84.3 (3)	85.0 (1)
90	Mild	73.5 (2)	76.0 (2)	75.3 (3)	73.5 (2)					
93	Mild	82.7 (3)	78.5 (2)	84.8 (5)	83.0 (3)					
123	Mild	72.0 (2)	76.0 (2)	75.0 (2)	72.0 (2)					
91	Mild	84.5 (2)	88.0 (1)	90.0 (2)	86.0 (2)	Mild	85.5 (2)	.....	91.0 (4)	91.0 (2)
34	Mild	78.7 (3)	82.0 (1)	85.8 (8)	81.5 (2)	Severe	79.0 (1)	.....	87.8 (6)	81.0 (2)
140	Mild	73.7 (3)	73.0 (1)	83.0 (3)	73.5 (2)					
82	Severe	81.5 (4)	.....	81.8 (4)	79.5 (2)	Severe†	81.7 (3)	77.0 (1)	94.5 (6)	Died
26	Severe	77.0 (3)	76.5 (2)	77.7 (7)	75.0 (3)					
122	Severe	70.5 (2)	72.0 (2)	74.3 (3)	69.0 (2)	Severe	67.5 (2)	68.0 (1)	78.7 (3)	Died
117	Severe	77.0 (3)	77.5 (2)	85.3 (3)	80.0 (3)	Severe	77.5 (2)	73.0 (3)	82.3 (3)	77.0 (2)
128	Severe	77.0 (2)	79.0 (2)	86.0 (3)	78.7 (3)	Mild†	78.0 (2)	80.0 (1)	78.5 (2)	
35	Severe	75.0 (3)	77.0 (2)	84.5 (2)	Died					

* Each measurement was derived from several readings (see text).
† In this rabbit nephritis was reprecipitated by an injection of normal duck serum.

over two hundred days, and the urine continued to show large amounts of albumin until death. Definite hypertension was present in rabbit 82 and in rabbit 22 (fig. 2).

RESULTS [30]

To determine the trend of blood pressure in the nephritic rabbits it was first necessary to ascertain the degree of variation in blood pressure from measurement to measurement in the normal animal. To obtain this information, the average of the blood pressure measurements for each animal in the control period was determined. The standard deviation of the individual blood pressure measurements from the respective averages of such measurements was then computed. The

TABLE 2.—*Differences in Mean Systolic Blood Pressures from the Control Period to the Period of Active Nephritis*

Primary Nephritis			Reprecipitated Nephritis		
Rabbit	Severity of Nephritis	Change, Mm. Hg	Rabbit	Severity of Nephritis	Change, Mm. Hg
121	Mild	—2.5	92	Mild	—0.2
95	Mild	—1.2	128*	Mild	+0.5
220	Mild	—0.5	121	Mild	+1.0
92	Mild	+1.0	91	Mild	+5.5
90	Mild	+1.8			
93	Mild	+2.1	21	Severe	+1.2
123	Mild	+3.0	17	Severe	+4.5
91	Mild	+5.5	117	Severe	+4.8
34	Mild	+7.1	34	Severe	+8.8
140	Mild	+9.3	22*	Severe	+8.8
			122	Severe	+11.2
82	Severe	+0.3	82*	Severe	+12.8
26	Severe	+0.7			
122	Severe	+3.8			
117	Severe	+8.3			
128	Severe	+9.0			
33	Severe	+9.5			

* In this rabbit nephritis was reprecipitated by an injection of normal duck serum.

resulting figure indicated not only the actual variations in blood pressure from day to day in the animals but also the degree of accuracy of the method used to determine the blood pressure. It was found that the standard deviation of measurements from the mean was 1.97 mm. of mercury in the control period. Thus significance could probably be attached to any measurement figure varying more than 3.94 mm. of mercury from the mean of the control period.

By a similar process of computation it was found that the variation from the mean was slightly increased (2.72 mm.) in the incubation period and further increased (3.30 mm.) in the active period and that in the recovery period the variability of measurements decreased to

30. The statistical analysis was prepared under the direction of Dr. E. L. Crosby Jr., of the statistical departments of the Johns Hopkins University and Hospital.

1.64 mm. These figures indicate that the blood pressure was more labile in the incubation and active periods of the nephritis than in the control period.

The average of the measurements for each animal in each period is shown in table 1.

It is evident from these figures that no significant rise in blood pressure occurred during the incubation period of the disease and that in most instances the figures for the recovery period closely approximated those for the control period. The differences in average blood pressures in the control period from those of the active period are shown in table 2.

It is apparent that in many animals the level of blood pressure was not significantly higher in the active than in the control period. Slight elevations were more frequently present in the severely nephritic rabbits than in those in which the disease was mild. The general trend was about the same in primary nephritis as in nephritis reprecipitated by nephrotoxic duck serum. An elevation in blood pressure was most clearly apparent in rabbits 22 and 82, in which the nephritis was reprecipitated by an injection of normal duck serum. From a detailed statistical analysis it is possible to state that the small rise in blood pressure observed in some of these animals could not have occurred as a result of chance alone.

SUMMARY

Experimental nephritis has been produced in rabbits by injecting anti-rabbit-kidney duck serum. Blood pressure was determined in 19 rabbits through thirty courses of nephritis. Blood pressure was more labile during the active period of nephritis than in the control period. No significant elevation in blood pressure occurred between the time of injection of the nephrotoxic serum and the appearance of the clinical nephritis. A small rise in blood pressure occurred during the active period of the nephritis in some of the animals, but this was not an invariable manifestation of the disease. These slight elevations of blood pressure were more frequently observed in severely nephritic animals than in those in which the disease was mild.

Changes in blood pressure were essentially the same in the primary attack of nephritis as in the nephritis subsequently induced by injection of nephrotoxic duck serum.

A moderate elevation in blood pressure was most clearly apparent in 2 rabbits in which severe nephritis was reprecipitated by injection of normal duck serum. It persisted more than two hundred days in 1, a rabbit in which the nephritis had become chronic.

PATHOLOGIC AND HISTOLOGIC CHANGES FOLLOWING ORAL ADMINISTRATION OF SULFAPYRIDINE

WITH A SHORT NOTE ON SODIUM SULFAPYRIDINE

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HARRY ROBINSON
RAHWAY, N. J.

Recently we reported the occurrence of urinary concretions in the rat, rabbit and monkey, consisting mainly of acetyl sulfapyridine, following oral administration of sulfapyridine (2-[paraaminobenzenesulfonamido]-pyridine).[1] Similar findings were reported by Gross, Cooper and Lewis [2] in experimental studies on rats. Previous to the foregoing reports, Oakley [3] in a short note on poisoning in mice due to "prontosil" (form not specified) reported the presence of "prontosil" crystals in the urinary bladder and gallbladder as well as concretions in the straight and convoluted tubules. Stewart, Rourke and Allen [4] observed the appearance of sulfanilamide crystals in the urine of patients and suggested the possibility of stone formation in the urinary tract. Laurence [5] was the first to report a case in which a patient experienced sharp pain in the lower right quadrant of the abdomen followed by gross hematuria, after administration of sulfapyridine and attributed this to the acetylated derivative. One of us (W. A.) has observed 2 similar cases in which the findings were highly, but not conclusively, suggestive of urolith effects. The presence of crystals in the urine of patients receiving sulfapyridine has already been reported by Stokinger.[6]

From the Laboratories of the Newark Beth Israel Hospital and the Merck Institute of Therapeutic Research.

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5. Laurence, E. A., in International Review of Recent Advances in Medicine, Washington, D. C., Washington Institute of Medicine, 1939, vol. 5, p. 48.

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In this paper we wish to present a report of pathologic and histologic changes following oral administration of sulfapyridine. A short description of the effects of sodium sulfapyridine is also included.

The animals used in these experiments were maintained on a balanced diet with sufficient water. Certain details of the experimental technic used in this investigation have been reported by Molitor and Robinson [7] in a study concerning the toxicity of sulfanilamide.

EXPERIMENTAL RESULTS

Concretions were observed after the administration of a large single dose of sulfapyridine, but the results were more striking after repeated dosing on successive days. The dosage at which uroliths were formed varied greatly in different species but to a much lesser degree in individuals of the same species. Thus, after sulfapyridine had been administered orally, concretions were frequently found in rabbits given 10 to 15 Gm. per kilogram, in rats given 5 Gm. per kilogram and in monkeys fed 0.25 Gm. per kilogram. When smaller doses were given, the individual variation in a given species was increased. In mice and dogs urolith formation was not observed even after the feeding of doses as large as 20 Gm. per kilogram. It is interesting to note that the latter species acetylate the compound very little.

In the course of this investigation approximately 400 mice, 320 rats, 42 rabbits, 16 dogs and 48 monkeys were used.

PATHOLOGIC OBSERVATIONS

With the dosage necessary for each species, the pathologic results were essentially alike in the rat, rabbit and monkey, and therefore a single description will suffice for all of these species.

The urolith formation was often unilateral, occurring more frequently on the right side. If the animal was put to death approximately twenty-four hours after the administration of the drug, aggregates of these crystals were often found in the ureter, especially at the level of the bony pelvic brim. After five to ten days of feeding the concretions were frequently found near the ureterovesical junction (fig. 1) or at times in the bladder. The calculi were soft and very friable, breaking up into minute fragments on slight pressure. Most of the concretions were irregularly ovoid but at times were elongated and formed a cast of the ureter. In animals killed during the early part of the feeding period the ureter was dilated and thinned out. Later it was indurated and in some instances markedly hemorrhagic. The kidney became edematous and enlarged to one and a half to two and a half times its

7. Molitor, H., and Robinson, H.: J. Pharmacol. & Exper. Therap. **65**:405, 1939.

normal size (fig. 1). At this time the kidney and ureters were found to be intimately bound with the periureteral tissues. Bloody urine was found within the dilated portion of the renal pelvis and ureter. In some instances an amorphous aggregate of crystals and fibrin completely filled the renal pelvis and extended upward into the papillary ducts of the kidney (fig. 2) but did not extend downward beyond the ureteropelvic junction. In these circumstances the ureter appeared normal.

Fig. 1.—Urinary system from a monkey which received 14 Gm. of sulfapyridine per kilogram for five days and died on the fifth day. The blood urea nitrogen was 82 mg. per hundred cubic centimeters. Calculi were found at the ureterovesical junction, with hydroureter, hydronephrosis and edematous enlargement of the kidney. Compare this with the normal kidney and ureter at the left.

The bladder was often edematous, especially in the region of the trigone. The ureterovesical orifices were prominent because of the lipping and congestion of the surrounding tissue.

Urea nitrogen was determined by the method of Van Slyke on the blood of the monkeys. All showed increased retention, ranging from a slightly elevated level to 100 mg. in 100 cc. of blood.

HISTOLOGIC OBSERVATIONS

Kidney (Rhesus Monkey).—The kidney revealed degeneration varying from mild to most severe, especially in the proximal convoluted tubules. With larger doses, the proximal and distal convoluted tubules could not be differentiated from each other. The cytoplasm of the cells forming these tubules was coarsely granular and occasionally vaculolated. Hyaline droplet degeneration was rare. The lumens of the

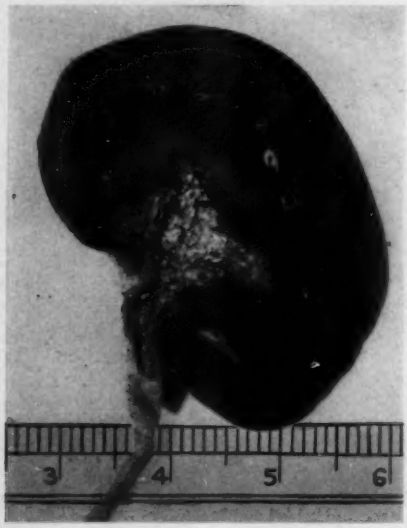

Fig. 2.—Kidney of a monkey receiving 5 Gm. of sulfapyridine per kilogram for six days. The blood urea nitrogen was 80 mg. per hundred cubic centimeters. Amorphous aggregates of crystals and fibrin completely fill the renal pelvis and extend into the papillary ducts.

tubules contained globoid eosinophilic bodies. The loops of Henle were markedly distended and contained, in addition to a few globular elements similar to those found in the convoluted tubule, a coagulum which was eosinophilic with hematoxylin and eosin and blue with azocarmine. In some instances the cells lining the parietal layers of Bowman's capsule were covered by cytoplasm in a cuboid form, in which a nucleus was rarely observed; when one was present, the cell closely resembled a cell of the proximal convoluted tubules (fig. 3*A*). These cells stained red with azocarmine, and transitions between these and the globular red-

staining bodies found in Bowman's space were noted. Apparently these are identical with the globoid bodies described in the tubular lumens. With azocarmine the glomeruli showed marked thickening of the basement membrane of the glomerular tuft (fig. 3 *B*). When doses of sulfapyridine insufficient to produce concretions were administered, the aforementioned degenerative changes were also found, though usually not as pronounced. Following excessive dosage, the glomeruli were markedly distended and contained, in addition to the cell debris, a coagulum similar to that found in the loops of Henle. The corresponding glomerular tufts were compressed, and the capillaries were collapsed and bloodless (fig. 3 *C*).

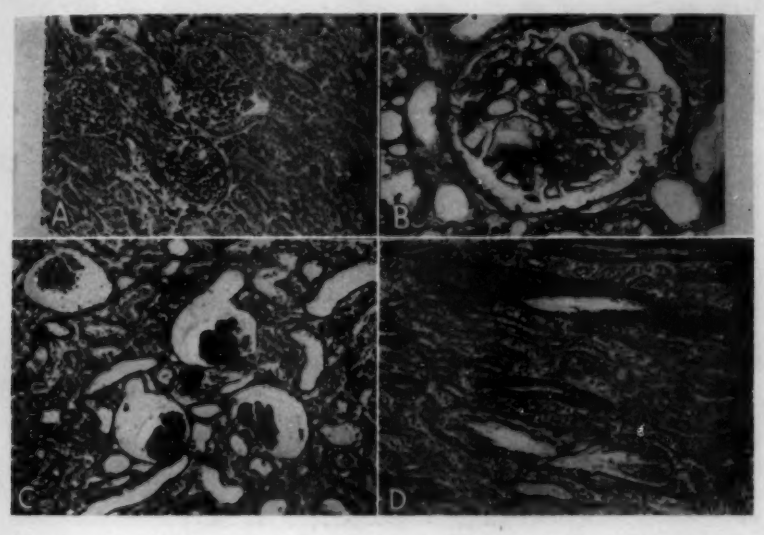

Fig. 3.—*A*, parietal layer of Bowman's capsule covered by cytoplasm in cuboid form, apparently in continuity with the epithelium of the proximal convoluted tubule; × 90. *B*, azocarmine stain showing thickened basement membrane of the glomerular tuft; × 250. Note globoid eosinophilic bodies in Bowman's space. *C*, distended glomerular spaces with compression of glomerular tuft; × 90. *D*, fibrin scaffolding in papillary ducts showing spaces from which crystals were dissolved out in preparation of section; × 90.

The collecting tubules showed only slight to moderate distention. The lumens of the papillary ducts were filled with coagulum, and only a few contained an appreciable number of the globoid bodies described. Other collecting tubules and papillary ducts contained coagulum and fibrin with clefts corresponding to spaces in which crystals had been present (fig. 3 *D*). This was most frequently observed when the renal pelvis was filled with the crystals and fibrin. Many of the tubules con-

tained and were surrounded by a mantle of polymorphonuclear leukocytes, and rarely a foreign body type of giant cell was found between the tubules.

Pyelonephritis and purulent periureteritis and peripyelitis were also observed.

If the animals were fed over a long period or had been permitted to survive for long periods after ample dosage, calcium casts were found in the tubules (fig. 4 *A*).

In the cases in which calculi were present within the ureter, the ureter and the renal pelvis showed from slight to extremely pronounced

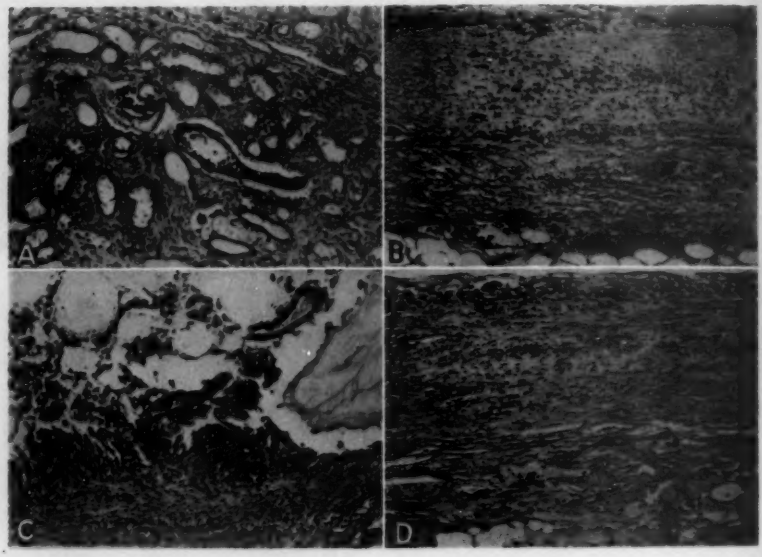

Fig. 4.—*A*, calcification of tubules; × 90. *B*, ureter showing edema, slight hemorrhage and a scattering of polymorphonuclear leukocytes; × 90. *C*, fibrin scaffolding in pelvis with clefts which contained crystals; × 90. *D*, edematous and hemorrhagic ureter.

edema of the lamina propria, with a moderate diffuse scattering of polymorphonuclear leukocytes (fig. 4 *B*). In isolated instances a phlegmon was found. The epithelium for the most part was intact, but an occasional erosion was observed.

In those cases in which the renal pelvis was found to be filled with an amorphous collection of fibrin and smaller calculi, the pelvis contained fibrinous material with clear clefts, which probably had the same significance as those described in the papillary ducts and collecting tubules (fig. 4 *C*). In places the transitional epithelium was absent, and in

these locations the fibrin rested directly on the lamina propria. The blood vessels in these regions showed extreme dilatation.

At times there occurred extremely hemorrhagic ureteritis and pyelitis, far in excess of that which might be expected from the presence of the corresponding calculus (fig. 4 *D*). Occasionally thrombi were encountered in the veins of the kidney. In some instances obliterating lesions (fig. 5 *B*) were found within lumens of veins which were not in close proximity to any of the inflammatory zones. Intraureteral hemorrhage was at times excessive, and in 2 cases the clotted blood caused urinary obstruction (fig. 5 *A*).

In some instances, after repeated feeding with doses insufficient to produce concretions the kidneys were edematous and revealed the glomerular and tubular changes described except that no "crystal casts" were found in the tubules. Because of this and the fact that, as men-

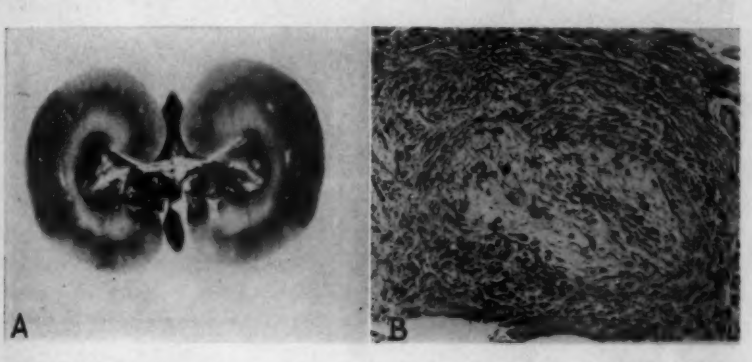

Fig. 5.—*A*, kidneys of a monkey receiving 5 mg. of sulfapyridine per kilogram for seven days, which was killed twenty-three days after the last feeding. The blood urea nitrogen was 11 mg. per hundred cubic centimeters. Note blood clot causing obstruction in the renal pelvis. *B*, obliterating endophlebitis; × 130.

tioned earlier, the hemorrhagic reaction may be extreme, the possibility must also be entertained that the drug may first produce parenchymal damage, and that subsequent to this the acetylated compound is precipitated from solution.

There are experimental data suggesting that the obstructing crystalline mass can either be redissolved or washed out. In a series of 5 monkeys, all of which received 4 Gm. of sulfapyridine per kilogram for ten days, 4 animals which were killed on the last day of feeding showed urolithiasis, while in the fifth, which was put to death seventy-nine days after the discontinuation of this treatment, no concretions were found. This animal exhibited, as evidence of transient urinary obstruction, slight thickening and dilatation of the ureters and pelvis. There were perirenal, peripelvic and periureteral adhesions, and the renal cap-

sule could not be stripped without tearing the renal parenchyma. The kidney showed scattered extensive collections of round cells, particularly in the subcapsular region (fig. 6*A*). Many of the tubules were filled with densely eosinophilic coagulum. Most of the glomeruli showed no appreciable damage. However, in the areas where the round cell infiltration was present, some were bloodless, others showed marked increase of cytoplasm, while others were undergoing fibrosis (fig. 6*B* and *C*). Bowman's capsule was occasionally thickened. One small vein showed recanalization. There was extensive round cell infiltration in the ureter and pelvis, and in places the epithelium dipped into the lamina propria (fig. 6*D*).

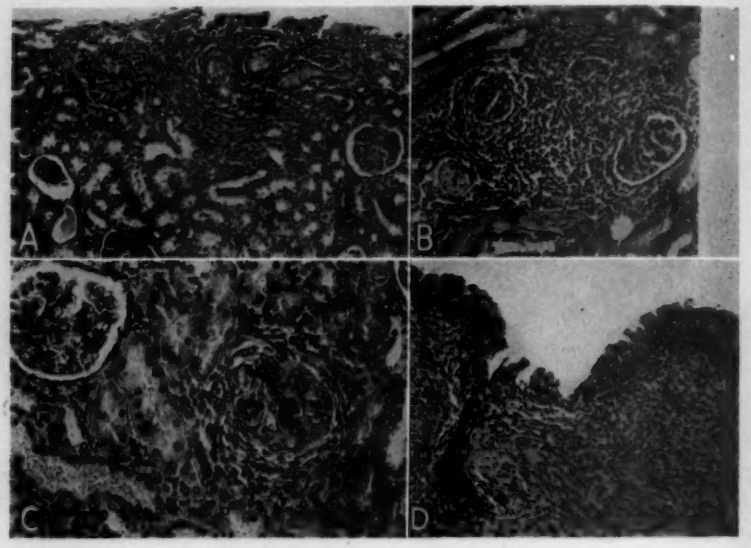

Fig. 6.—Sections of kidney of a monkey receiving 4 Gm. of sulfapyridine per kilogram for ten days, which was put to death seventy-nine days after the last feeding; × 90. No calculi were found. *A*, extensive round cell collections in the subcapsular region. The capsule could not be stripped without tearing the kidney substance. Note the dilated tubules filled with eosinophilic coagulum. *B* and *C*, areas showing round cell infiltration. Many glomeruli are bloodless, with increase in cytoplasm. *D*, subepithelial round cell infiltration in pelvis.

The uroliths ordinarily permit penetration by roentgen rays and therefore do not cast a shadow. However, calcium may be deposited about these concretions, which act as a nucleus, in which case the shell may become roentgen ray opaque.

Adrenal.—In the rat the adrenal was enlarged, and the cortex appeared gray. Histologically, the fascicular layer was markedly widened

and the cytoplasm in this area was deeply eosinophilic and in many instances showed no vacuolation and contained little lipoid. In the monkey, in the early stage the changes were similar to those in the rat but not as pronounced. Prolonged feeding did not result in greater changes.

There were occasionally calcific areas in the adrenal, but these were also encountered in the normal controls.

Liver.—At times, especially in the monkey, the liver showed separation of the capillaries from the liver cord cells, with granular material in the intervening spaces—similar to the "serous hepatitis" of Rossle-Eppinger. With Best's carmine stain, the liver cells were diffusely filled with glycogen.

Other Organs.—The stomach often showed marked congestion with an increase in mucous secretion. The heart did not show any significant changes. The cells lining the acini of the thyroid were cuboid. The colloid, especially near the cell periphery, was pale staining, suggesting mild hyperplasia. The bone marrow showed an increase in polymorphonuclear leukocytes. The response of the marrow will be reported later in collaboration with L. Goldman and W. Sampson. The spleen showed considerable deposition of iron, especially marked in the perifollicular zone. Erythrophagocytosis was not uncommon in the pulp. The pulp usually showed marked congestion. Lymphoid hyperplasia was variable. The lymph nodes were usually hyperplastic and showed marked proliferation of the lining cells of the sinuses.

COMMENT

From the observations described one cannot conclude definitely whether the precipitation of the acetylated sulfapyridine in the urine is always primary or whether it is dependent on initial degenerative changes in the renal parenchyma or on a combination of both. Since the degenerative changes are found without urolith formation in some instances, one may safely assume that the former changes are not dependent on the formation of concentrations. The possibility remains that the formation of uroliths may at times be initiated by the degenerative renal or vascular changes; on the other hand, it may be quite independent of these changes.

With the oral administration of sodium sulfapyridine, smaller doses were necessary for urolith formation in the rat, rabbit and monkey. Histologically the degenerative changes in the kidney were more pronounced than those found in animals fed sulfapyridine. In addition the mucous secretion of the stomach was very striking, and in isolated

instances the rabbit showed acute diffuse gastritis. When this drug was administered rectally, a hemorrhagic reaction with some necrosis of the mucosa was frequently encountered.

SUMMARY

Urolithiasis occurs after the feeding of sulfapyridine to monkeys, rabbits and rats. The concretions can be either redissolved or washed out. It cannot be ascertained whether the formation of the urolith is always an independent precipitation process or whether it is at times dependent on primary degenerative or vascular changes in the kidney.

PLASTIC STUDIES IN ABNORMAL RENAL ARCHITECTURE

V. THE PARENCHYMAL ALTERATIONS IN EXPERIMENTAL HYDRONEPHROSIS

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BROOKLYN

In his classic study of the problem of hydronephrosis Ponfick,[1] recognizing the inadequacy of the method of histologic section, summarized the difficulties that he encountered in the identification of structures and in the interpretation of the topographic interrelations of the altered renal parenchyma in the following words:

> Denn infolge der tiefgreifenden Umwälzungen, die sich an den Tubulis verschiedenster Art vollzogen haben, sind deren Eigentümlichkeiten nachgerade dermassen verwischt, dass man wohl im Zweifel bleiben kann, ob man contorti oder recti, vielleicht sogar Henlesche Schleifen vor sich habe. (For, owing to the profound changes which have taken place in the various types of tubuli, their characteristics are blurred to such an extent that one may remain in doubt whether one has to do with convoluted or with straight tubules or even with Henle's loops.)

As has been demonstrated by Oliver and his co-workers,[2] the method of microdissection particularly favors the elucidation of such problems, and on his suggestion the method has been applied to the study of the effect of ureteral ligation on the rabbit kidney. In the discussion that follows it will become apparent that many of the assumptions that have been made concerning histologic pictures in hydronephrosis are without support when viewed in the reality of three dimensions.

That the technic of microdissection previously had been applied to this problem [3] appeared not to contraindicate its further use, as one does not find in the extensive subjective interpretations of this worker's fragmentary presentations the essential value of the method, which is the demonstration of actual objects in structural continuity and their topographic relations.

This work was done with the support of the Josiah Macy Jr. Foundation.

From the Department of Pathology of the Long Island College of Medicine, the Hoagland Laboratory.

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MATERIAL AND METHODS

Rabbits were the animals of choice because of the simplified form of their kidneys and because normal rabbit kidneys have been studied intensively by the method of microdissection by Peter.[4] The animals used included both males and females and were usually between 6 months and 1 year old. Under ether anesthesia their left ureters were ligated and cut about 1 cm. below the ureteropelvic junction without interference with the renal vessels. Fourteen animals were put to death at intervals of from one to two hundred and thirty-one days. Six additional animals, about 6 months old, were similarly treated and put to death at intervals

Data on Experiments

Rabbit	Weight of Rabbit, Gm.	Duration of Obstruction, Days	Weight of Kidney, Gm.	Measurements of Kidney	Volume of Pelvic Fluid, Cc.
6	2,360	1	R 8.2 L 14.6	3.4 × 2.5 × 2.0 4.1 × 2.9 × 2.5	4
20	1,870	3	R 8.0 L 17.5	3.5 × 2.5 × 2.0 4.5 × 3.2 × 2.8	6
21	2,270	6	R 8.9 L 26.9	3.5 × 2.5 × 2.0 5.0 × 3.5 × 3.0	8
14	2,380	10	R 9.2 L 25.1	3.4 × 2.4 × 2.1 5.0 × 3.7 × 2.8	9.6
7	2,210	14	R 7.8 L 23.6	3.3 × 2.2 × 2.1 4.2 × 2.7 × 2.4	10
15	2,080	21	R 7.0 L 29.0	3.0 × 2.0 × 1.3 4.0 × 2.6 × 2.1	12
16	2,300	28	R 7.4 L 34.2	3.3 × 2.0 × 1.3 4.7 × 3.1 × 2.2	18.4
17	2,340	36	R 8.1 L 45.8	3.2 × 2.3 × 1.5 5.6 × 4.0 × 3.3	35
8	2,450	46	R 8.29 L 58.0	3.6 × 2.4 × 1.6 6.3 × 4.6 × 4.2	46
19	2,750	61	R 9.3 L 54.4	4.0 × 2.5 × 2.0 6.0 × 4.0 × 4.0	40
9	2,400	90	R 8.0 L 21.0	3.8 × 2.5 × 2.0 4.5 × 3.8 × 2.9	15
13	2,470	114	R 8.4 L 17.3	3.7 × 2.4 × 2.0 4.5 × 3.6 × 3.0	12
10	2,900	163	R 8.4 L 14.1	3.6 × 2.5 × 2.1 4.0 × 3.2 × 2.6	10
11	2,550	231	R 8.1 L 8.5	3.5 × 2.5 × 2.0 3.2 × 2.4 × 2.3	6

of from three to six weeks. Another animal was excluded from the series because a staphylococcic infection of the obstructed kidney was discovered after five weeks, but the other hydronephrotic kidneys remained free from infection.

After the kidneys had been weighed and measured and the pelvic fluid removed (table), the organs were sectioned longitudinally and then cut transversely, the first blocks being taken through the center of the papillary area. Blocks were fixed in Zenker's fluid for routine hematoxylin and eosin stains and for iron stains, in solution of formaldehyde U. S. P. for fat stains and in Kolster's fluid for the Altmann stain for mitochondria. The remainder of the organ was placed in solution of formaldehyde until it could be dissected.

4. Peter, K.: Untersuchungen über Bau und Entwicklung der Niere, Jena, Gustav Fischer, 1927.

Thin blocks of tissue then were cut through the papilla adjacent to that used for the routine microscopic sections. These blocks were allowed to stand in concentrated hydrochloric acid at room temperature until softened sufficiently for dissection; they were then washed in several changes of water and placed in distilled water in a Stender dish. The tissue was then dissected under a binocular microscope with the aid of needles. The nephrons were examined at magnifications of 33 and 100 while supported in water and were photographed stereographically at a magnification of 8 times. From the plates, transparencies were prepared and mounted for study in the stereoscope, and from these the final drawings of figures 10 to 42 were made. The location of each specimen photographed, together with notes on the topographic relations, were recorded at the time on an outline drawing of the block of tissue being examined, and from these data, supplemented by the study of histologic sections, the topographic arrangements of figures 43 to 54 were prepared. For purposes of description the transverse sections of the kidney are divided into central, intermediate and lateral areas. It will be seen that this division is not arbitrary but is made in accordance with distinctive structural alterations developing within these three zones.

GROSS STRUCTURAL CHANGES

In general, the macroscopic changes in renal architecture resulting from ureteral ligation are well known; however, several features of the gross findings merit particular attention.

As early as three days after ligation of the ureter the papilla of the rabbit kidney is literally torn apart (fig. 1), and shortly thereafter the ducts of Bellini are no longer to be found. At the site of the papilla there develops an irregular oval depression, and from its margins radiate the interrupted collecting tubules (fig. 2). With the advance of the lesion the depression becomes larger as the tubules are increasingly drawn apart by the accumulation of the pelvic fluid. With the passage of time these interrupted tubules become enclosed by connective tissue and covered by epithelium, so that in the twenty-eight day specimen the continuity of the pelvic and tubular epithelium has been restored, and in the sixty-one day specimen the collecting tubules are found separated from the pelvis by connective tissue and the pelvis is lined by an uninterrupted sheet of epithelial cells (fig. 3).

The parenchymal weights of obstructed kidneys increase for periods up to and including sixty-one days and beyond this interval decrease progressively to reach a figure below the original (table). The rate of gain is variable. It is greatest in the first few weeks of obstruction. This finding corresponds with that of Ponfick [1] and, as he described, seems due to congestion, edema and accumulation of fluid in dilated tubules.

In the first few weeks, when the secretory capacity of the parenchyma may reasonably be assumed to be best maintained, the rate of accumulation of pelvic fluid is relatively slow and inconstant. The fluid is yellowish or only slightly blood tinged, is moderately turbid and

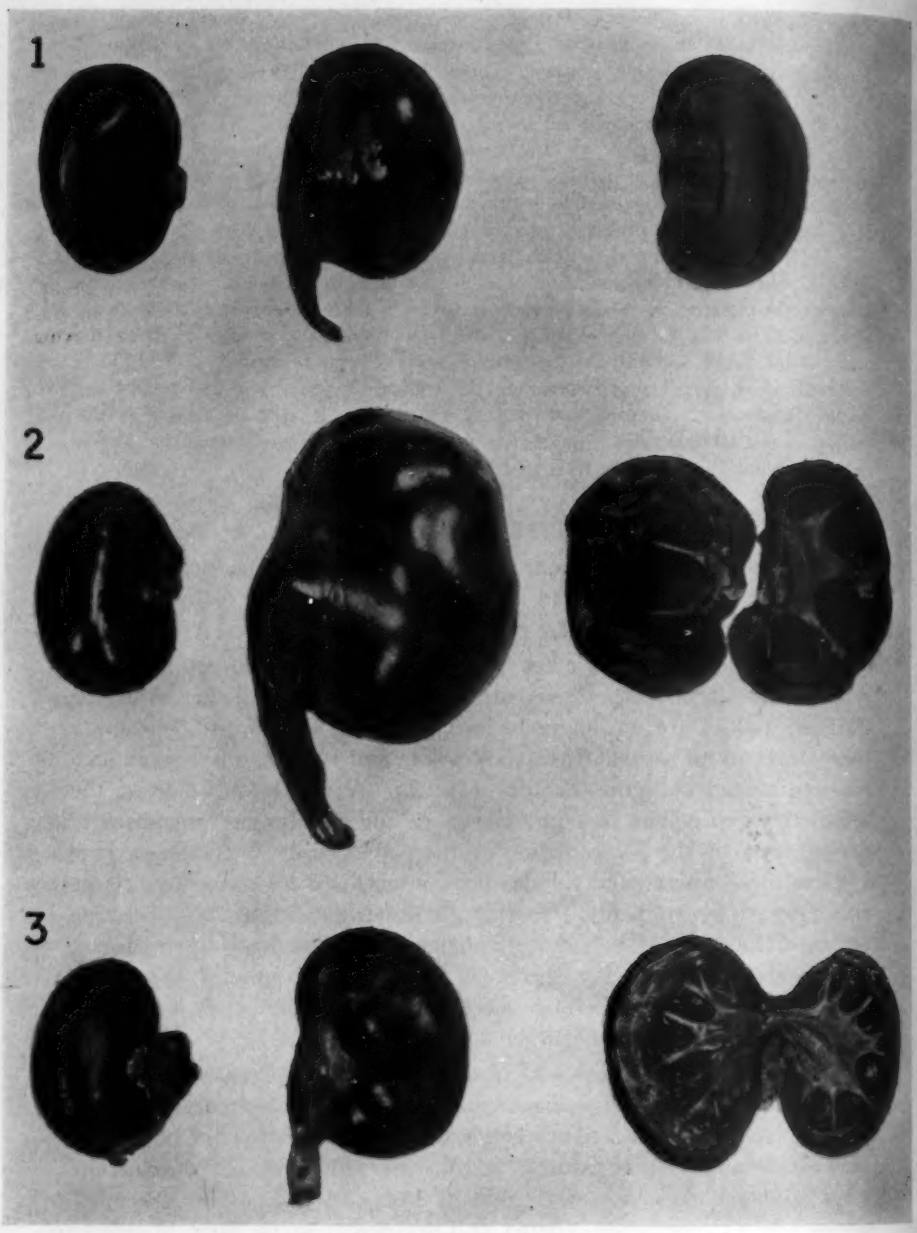


Fig. 1 (rabbit 20).—Three days' obstruction.

Fig. 2 (rabbit 8).—Forty-six days' obstruction.

Fig. 3 (rabbit 9).—Ninety days' obstruction. The three figures show normal and hydronephrotic kidneys and longitudinal sections of the latter showing (1) the variation in volume of the organs, (2) flattening, splitting and tearing of the papilla and (3) newly proliferated uninterrupted pelvic epithelium covering the site of the obliterated papilla.

contains small numbers of desquamated epithelial cells, leukocytes, hyaline casts and considerable albumin. Between the fourth and seventh week of ureteral occlusion there is a rapid increase in volume of pelvic fluid, and it changes in character, becoming frankly bloody.

On sedimentation, one third of the pelvic fluid of the thirty-six day specimen is found to consist of packed erythrocytes. The forty-six day specimen yields the maximum volume of pelvic fluid, and this contains a slightly increased proportion of packed erythrocytes. The blood in these instances is obviously fresh and becomes correspondingly less so in later specimens. There is only a slightly decreased volume of pelvic fluid in the sixty-one day specimen, but later the fluid gradually decreases in volume (table) and becomes thinner and lighter in color, although it remains somewhat turbid.

PARENCHYMAL ALTERATIONS

Glomeruli.—(*a*) Microscopic Sections: Owing to compression of the capillary tuft, Bowman's space is enlarged and frequently contains a slight amount of coagulated fluid and rarely an occasional erythrocyte. In some examples the tuft (figs. 4 to 9) shows cleftlike spaces between adjacent capillary loops, and throughout the series the latter are relatively bloodless. In the forty-six day specimen the parietal layer of Bowman's membrane is slightly thickened, and this change is somewhat more marked in later stages (fig. 9), but connective tissue proliferation and hyalinization within the tuft are not seen until two hundred and thirty-one days of obstruction and then are inconspicuous in most glomeruli (fig. 9). There is no appreciable increase in the external diameter of glomeruli save in certain cystic dilatations of the space of Bowman to be described later. These occur in the forty-six, sixty-one and ninety day specimens, and histologically the glomeruli present no structural peculiarities save their increase in size and the thickening of Bowman's membrane mentioned.

(*b*) Microdissection: Save in rare instances, in later stages the volume of the glomeruli is not increased; on the contrary, reduction in glomerular volume is evident in twenty-four hours and in general is maintained throughout the period of the experiments. Although in the latest stage a further reduction in volume is apparent, there is nothing to suggest the complete disappearance of glomeruli during the two hundred and thirty-one day interval of this study.

In the early stages variability in size and shape is greater than normal, and although examples of concomitant decrease in size of glomeruli and their proximal convoluted tubules are found, many of the atrophied proximal convoluted tubules too minute for dissection possess glomeruli as large or even larger than those associated with the best preserved proximal convoluted tubules of the same specimen (figs. 23 and 26).

Fig. 4 (rabbit 20).—Three days' obstruction. Dilatation of tubular lumens and flattening of epithelial cells in the inner part of the cortex of the central area. Magnification, 150.

Fig. 5 (rabbit 14).—Ten days' obstruction. Increasing dilatation of tubular lumens with beginning distention of collecting tubules and of distal convoluted tubules and variable atrophy of proximal convolutions in the inner part of the cortex of the central area. Magnification, 150.

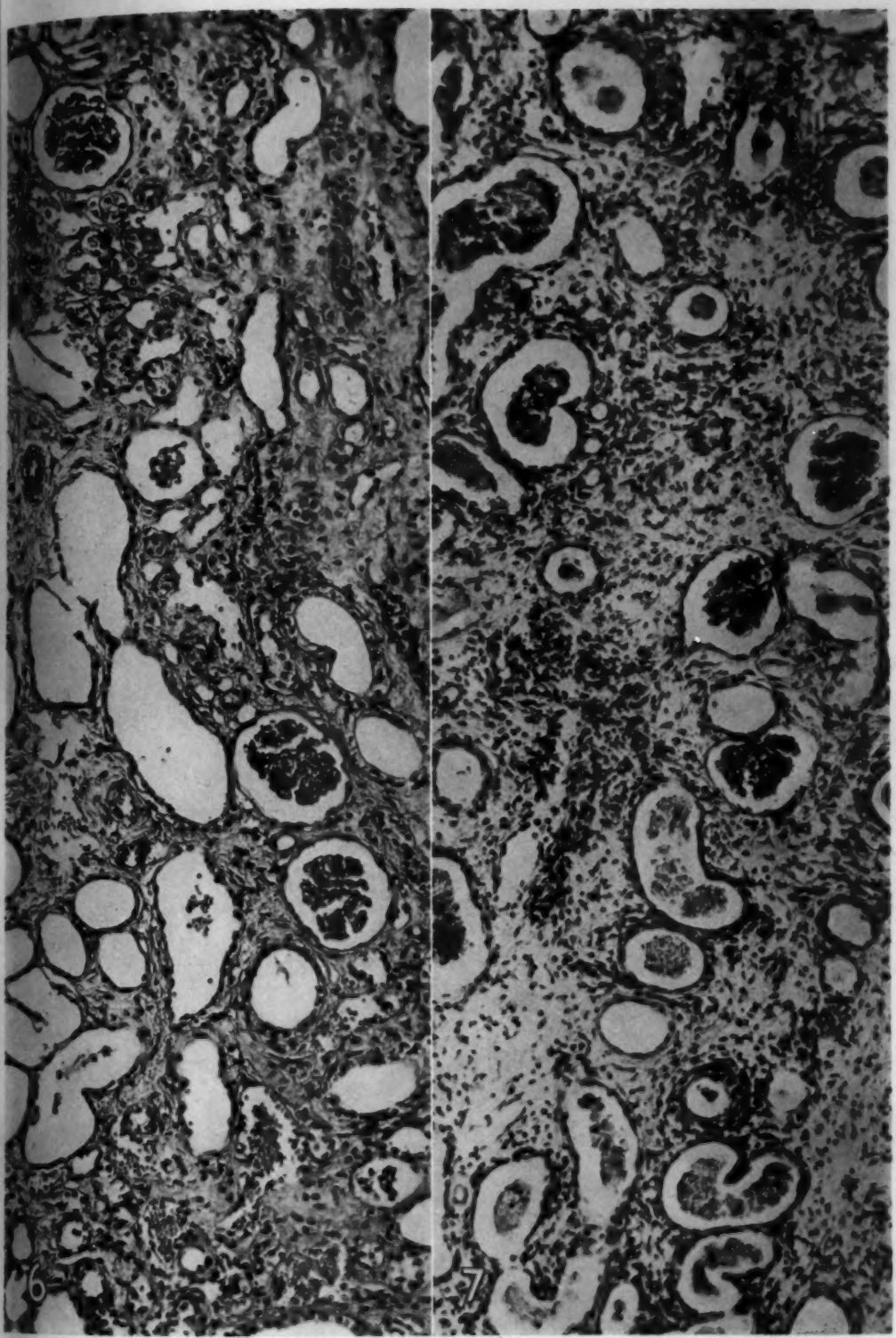


Fig. 6 (rabbit 16).—Twenty-eight days' obstruction. Increased interstitial change and epithelial atrophy in the cortex of the central area. The dilated tubules are distal convoluted tubules and collecting tubules. Note the repeated cuts across one of the few identifiable proximal convoluted tubules just below the uppermost glomerulus. Magnification, 150.

Fig. 7 (rabbit 17).—Thirty-six days' obstruction. The epithelial atrophy, leukocytic infiltration and connective tissue proliferation are more marked in the cortex of the central area. Note the blood accumulated in tubules known from dissection to be distal convoluted tubules. Magnification, 150.

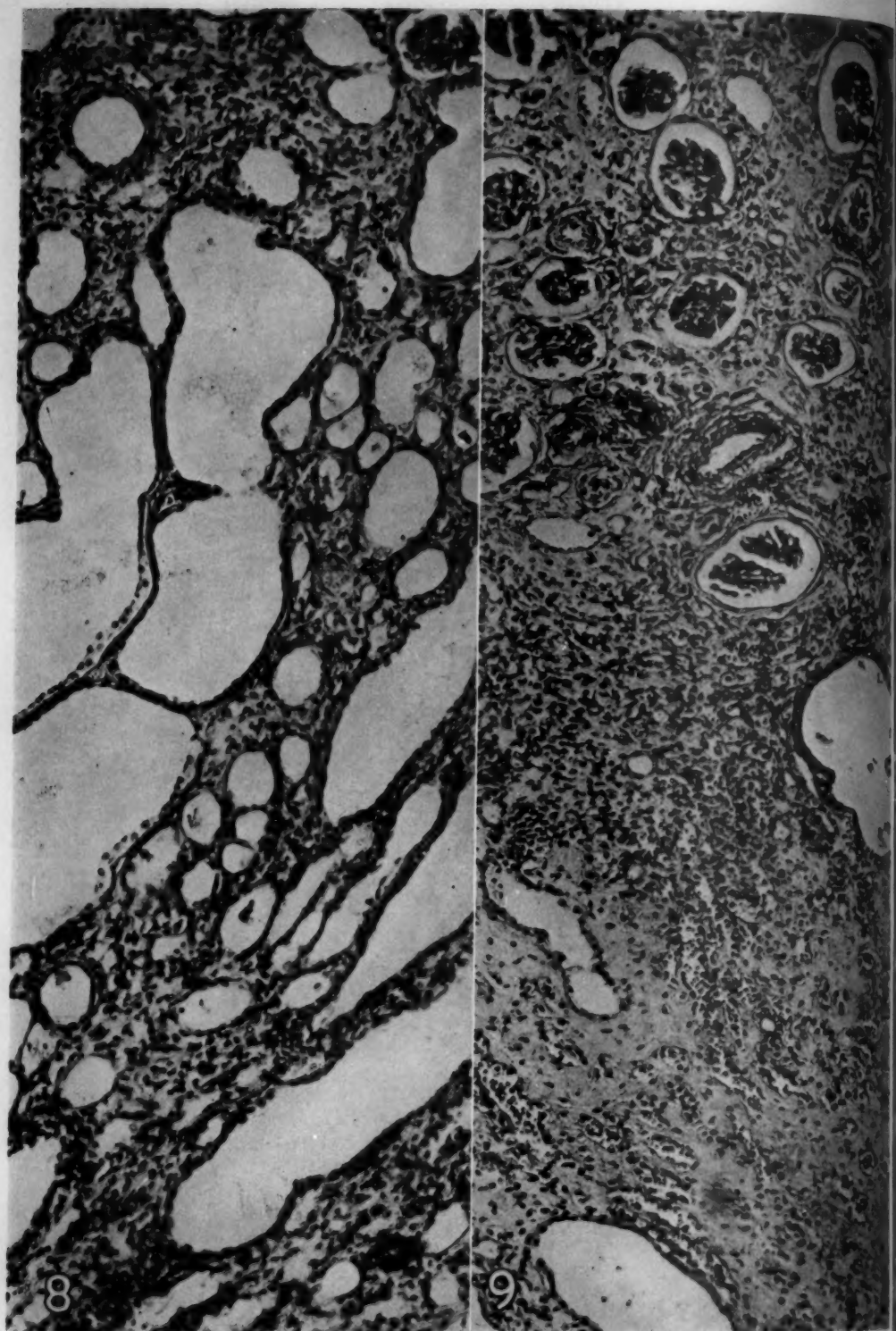


Fig. 8 (rabbit 17).—Thirty-six days' obstruction. Interstitial fibrosis, leukocytic infiltration and dilatation of collecting tubules in the outer portion of the medulla of the central area. These collecting tubules are narrowed at the corticomedullary boundary. Magnification, 150.

Fig. 9 (rabbit 11).—Two hundred and thirty-one days' obstruction. The connective tissue between the concentrated glomeruli is infiltrated by leukocytes and is practically devoid of identifiable tubular remnants. The capillary tufts present, for the first time in this series, fibrosis and hyaline change, but this is infrequent and slight. Note the cystic collecting tubule of the outer part of the medulla. Magnification, 150.

In the forty-six and sixty-one day specimens and especially in the ninety day specimen (fig. 32) cystic dilatation of Bowman's space is marked in certain glomeruli. Some of these cystic glomeruli lack proximal convoluted tubules, but in no case does the cystic dilatation extend into the proximal convoluted tubules, which commonly appear as minute twigs opposite the vessel pole.

The "concentration" of glomeruli due to their approximation is evident early and is especially apparent in the dissected specimen in the later stages, when the cortex is largely composed of glomeruli separated only by connective tissue fibrils and minute unidentifiable tubular remnants.

Proximal Convoluted Tubules.—(*a*) Microscopic Sections: From the first day of obstruction marked change is evident in the proximal convoluted tubules (fig. 4). The lumens of some are increased in caliber, and their epithelial cells are variably flattened against the basement membranes; other tubules are compressed, and in some the epithelial cells present marked parenchymatous degeneration or considerable vacuolation of their cytoplasm. Mitotic figures are not uncommon among the epithelial cells of these elements in the three and six day specimens but are not seen later. At no time is there evidence of bleeding into the proximal convoluted tubules.

In the ten day specimen there is apparent a topographic distribution of the atrophy that will be considered later. In somewhat more advanced stages, with progressive atrophy of cortical tubules and increasing proliferation of connective tissue, there develops the admitted difficulty in the identification of the tubules in microscopic sections. However, small "groups" of markedly atrophic proximal convoluted tubules are easily identifiable after four or five weeks (fig. 6).

(*b*) Microdissection: In the material of this study hypertrophy of the proximal convoluted tubule was not encountered nor was the external diameter of this element ever found increased over normal by dilatation. A perceptible shortening and a definite reduction in caliber are, however, evident in many of the proximal convoluted tubules after twenty-four hours' obstruction.

In the three and six day specimens the atrophy is considerably increased (figs. 11, 18 and 19) and the largest proximal convoluted tubule encountered is definitely atrophic and of increased fragility. The variation in caliber and in refractive qualities is segmental in character and irregularly placed, and adjacent proximal convoluted tubules commonly vary in size and in the degree of change at any particular level. The only change found localized within individual tubules with any constancy is a lesser decrease in refractive qualities of the initial portion of the tubule and increased thinness, delicacy and translucence of the

Explanation of Figures 10 to 16

Fig. 10.—Complete nephron and collecting tubules from a normal kidney. Magnification, 20.

Fig. 11 (rabbits 20 and 21).—Atrophy of the proximal convolutions in the central area after three and six days' obstruction. Note the reduction in size of the glomeruli. Magnification, 20.

Fig. 12 (rabbit 7).—Extreme atrophy of glomeruli and proximal convolutions in the lateral area after fourteen days' obstruction. Magnification, 20.

Fig. 13 (rabbit 15).—Proximal convolution from the intermediate area after twenty-one days' obstruction. Contrast the initial and terminal portions. Magnification, 20.

Fig. 14 (rabbit 16).—Well preserved proximal convolution from the intermediate area and the only dissectable proximals from the central area after twenty-eight days' obstruction. Note similar glomeruli on both types of nephrons. Magnification, 20.

Fig. 15 (rabbit 20).—Duct of Bellini and lower collecting tubules after three days' obstruction, showing little change. Magnification, 20.

Fig. 16.—Duct of Bellini and lower collecting tubules from a normal kidney. Magnification, 20.

Figures 10 to 16

EXPLANATION OF FIGURES 17 TO 22

Fig. 17 (rabbit 14).—A stretched collecting tubule to the lateral area after ten days' obstruction. Magnification, 20.

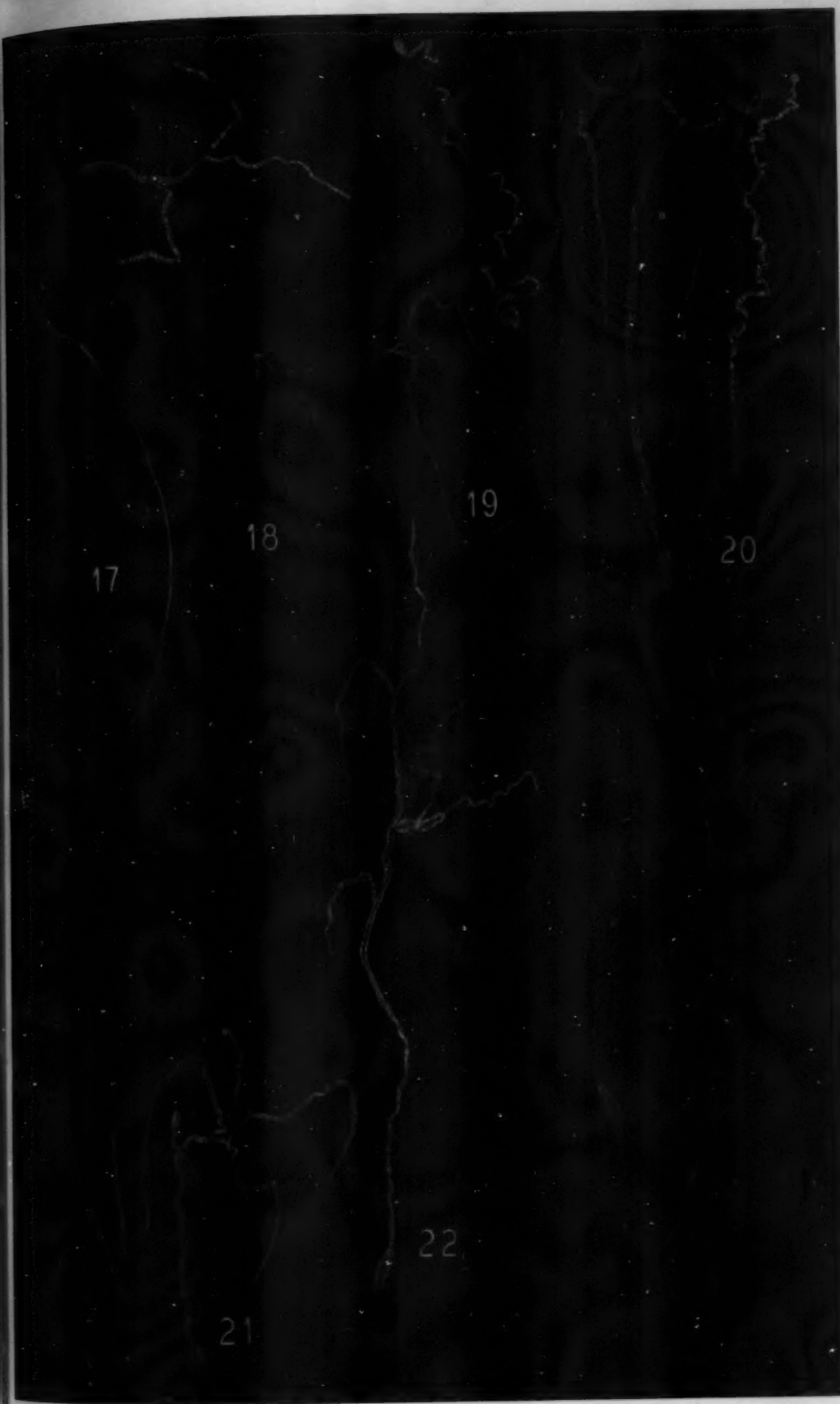
Fig. 18 (rabbit 21).—An atrophic proximal convolution from the central portion after six days' obstruction. Contrast the length of this tubule with the tubule on the right in figure 11, which is from the same zone of the same kidney. Magnification, 20.

Fig. 19 (rabbit 21).—A fairly large proximal convolution from the lateral area after six days' obstruction. Note the variability from one portion to another. Magnification, 20.

Fig. 20 (rabbit 14).—Dilatation of collecting tubules in the central area after ten days' obstruction. Magnification, 20.

Fig. 21 (rabbit 15).—Obliteration of distal convolutions in the central area and the formation of budlike structures on the collecting tubule after twenty-one days' obstruction. Magnification, 20.

Fig. 22 (rabbit 15).—Cystic dilatation of the connecting piece after twenty-one days' obstruction. Magnification, 20.



Figures 17 to 22

terminal segment (fig. 13). In later stages this fragile, dilated terminal segment becomes molded by developing fibrosis, with the production of saccular deformities, and persists in some instances for several weeks after the remainder of the tubule has lost its identity as a dissectable structure (fig. 24).

At ten days a topographic distribution of the atrophy within the organ becomes evident (figs. 45 and 46). At the end of fourteen days the atrophy of many of the proximal convoluted tubules is marked (fig. 12), and in review of specimens, including that of twenty-one days, it appears that the rate of atrophy is greatest in the first week. At twenty-eight days dissectable proximal convoluted tubules are uncommon save in one certain area of the organ (figs. 23 and 25), and in the thirty-six day and subsequent specimens they are too atrophic for dissection save in this same limited region (figs. 28 and 29). In the ninety, one hundred and sixty-three and two hundred and thirty-one day specimens no dissectable proximal convoluted tubules are found, although a few relatively well preserved elements were present in the one hundred and fourteen day specimen (fig. 28).

Loops of Henle.—(*a*) Microscopic Sections: In the first weeks the loops of Henle commonly present a dilatation of their lumens with flattening of the epithelial cells and often contain coagulated fluid (figs. 4 and 5). Later certain of them are compressed by the dilatation of the collecting tubules between which they lie and also by the proliferating interstitial tissue. Some are also observed to extend into the margin of necrosis lining the fragmented papilla.

(*b*) Microdissection: The transition of the proximal convoluted tubule to the thin segment of Henle's loop is sharp and distinct and is consistently found in all the specimens isolated (figs. 11-14 and 23). The boundary between the *Zwischenstück* (middle piece) and the distal convoluted tubule is never as distinct as that between the proximal convoluted tubule and the thin limb, but the dilatation of the distal convoluted tubule to be described later never extends into the loop of Henle. Although the loop of Henle becomes increasingly thin and delicate and correspondingly more difficult to isolate as an intact structure, it can be traced with sufficient accuracy to indicate that it retains its identity without any extraordinary shortening through an interval of thirty-five days, after which it is usually impossible to separate the structure with any satisfaction from the connective tissue fibrils and atrophic tubular remnants. When Henle's loops were found to be compressed or even interrupted by pressure of adjacent dilated collecting tubules or by encroachment of connective tissue they were not found to be dilated proximal to the stenosis or occlusion. Indeed, an increase in the external diameter of a loop of Henle was never observed at any time during the development of the lesion (figs. 20, 21, 30 and 31).

Distal Convoluted Tubules.—(*a*) Microscopic Sections: After twenty-four hours' obstruction there is considerable dilatation of the lumens of the distal convoluted tubules with flattening of the epithelium against the basement membranes (figs. 4 and 5). Their lumens frequently contain coagulated fluid, which, however, does not appear as a cast capable of obstructing the lumen. In the first weeks there is little additional change save that the epithelial atrophy is more marked and that an actual increase in external diameter occurs. In the ten and twenty-one day specimens a few well preserved erythrocytes are present in the distal convoluted tubules in the central area of the organ.

After twenty-one days the distal convoluted tubules are not identifiable by their histologic appearance in sections with the exception of those in one particular region of the kidney. These can be recognized, in fact, only by reference to the appearances noted in dissected material, where after thirty-six days' obstruction they are found to be enlarged and filled with blood (fig. 7). Blood is not observed elsewhere in the kidney save in the associated collecting tubules. In all the later specimens blood is found in similar locations, with the red cells progressively fused and hyalinized and with granules of hemosiderin appearing within them.

(*b*) Microdissection: In the first few days of obstruction the contour of the distal convoluted tubules is unchanged, but they become increasingly more fragile and are of decreased refractive qualities. At six days there is an infrequent slight increase in external diameter, and this becomes somewhat more marked at ten and fourteen days (figs. 17 and 20). There may be variability in the degree of distention of the distal convoluted tubules joining one collecting tubule. The increase in external diameter in no case is found to extend into the ascending limb of Henle but frequently is continuous with that of the connecting piece and collecting tubule. The latter, together with the first part of the distal convoluted tubule in the twenty-one, twenty-eight and thirty-six day specimens, presents prominent local cystic dilatations highly irregular in distribution (figs. 21, 22, 27 and 31). The walls of these cystic structures are exceedingly thin and delicate, and some are found to end blindly as they are dissected from the connective tissue in which they are embedded.

At thirty-six days greatly increased distention and deformity of certain of the distal convoluted tubules are observed, and they are filled with blood. The latter is confined almost entirely to loops of the distal convoluted tubules, being present elsewhere, in small amount, only in the cortical portions of the corresponding collecting tubules (fig. 40). From forty-six days to two hundred and thirty-one days pigmented distal convoluted tubules are found in similar distribution. With increasing

EXPLANATION OF FIGURES 23 TO 33

Fig. 23 (rabbit 16).—Well preserved proximal convoluted tubule from the intermediate area after twenty-eight days' obstruction. Magnification, 20.

Fig. 24 (rabbit 16).—Terminal portion of a proximal convoluted tubule from the intermediate portion of the kidney after twenty-eight days' obstruction. Note the variability in caliber. Magnification, 20.

Fig. 25 (rabbit 17).—Increased atrophy of the proximal convolution in the intermediate area after thirty-five days' obstruction. Magnification, 20.

Fig. 26 (rabbit 16).—One of the very few dissectable atrophic proximal convolutions from the lateral area after twenty-eight days' obstruction. Magnification, 20.

Fig. 27 (rabbit 17).—Cystic dilatations of the distal convoluted tubules in the intermediate zone after thirty-five days' obstruction. Magnification, 20.

Fig. 28 (rabbit 13).—Atrophic proximal convolutions from the central area after one hundred and fourteen days' obstruction. Magnification, 20.

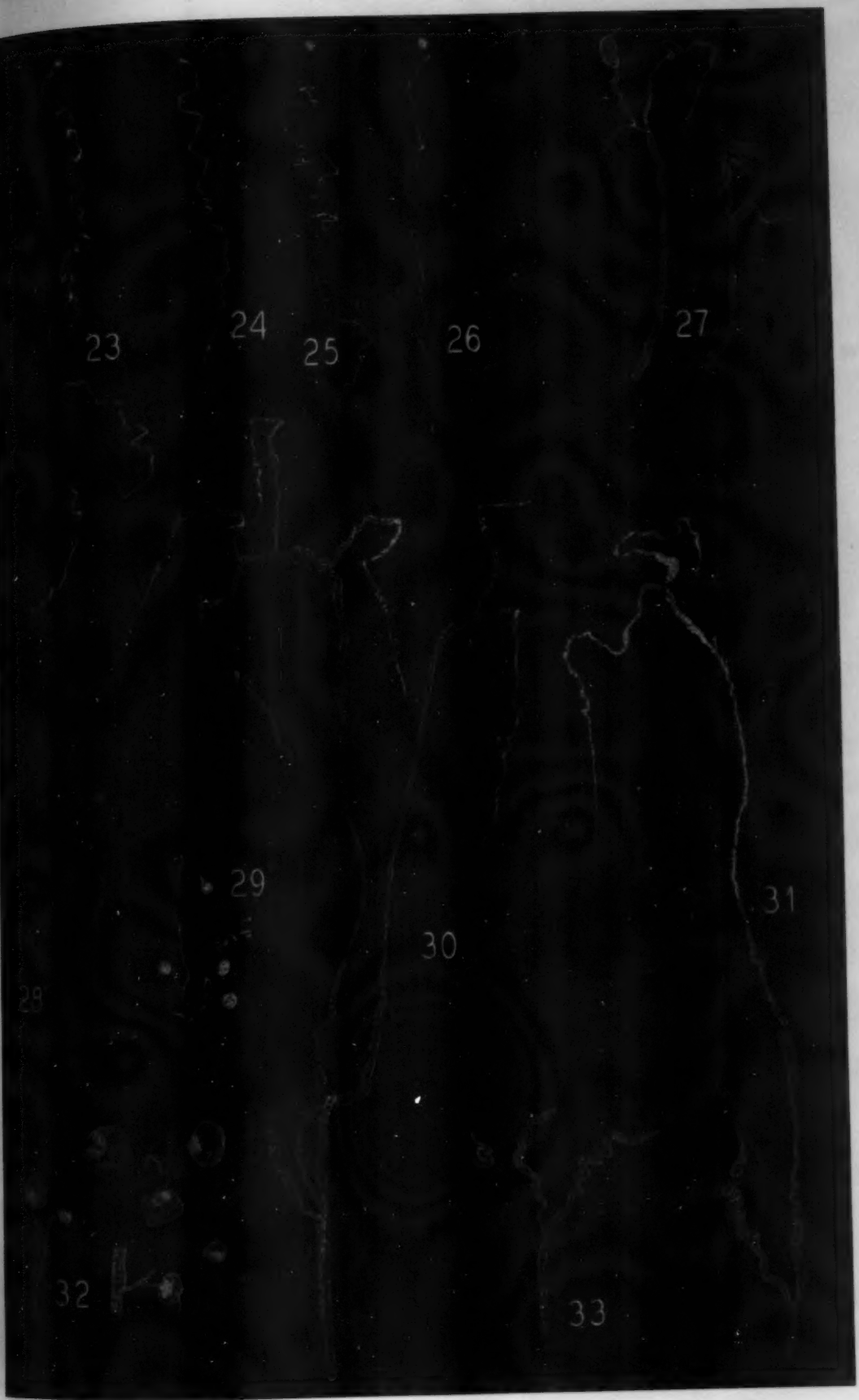
Fig. 29 (rabbit 8).—Atrophic proximal convolutions from the lateral area after forty-six days' obstruction. Magnification, 20.

Fig. 30 (rabbit 16).—Dilated and deformed central collecting tubules from the central portion after twenty-eight days' obstruction. Magnification, 20.

Fig. 31 (rabbit 16).—Dilated, deformed and interrupted collecting tubules from the central area after twenty-eight days' obstruction. Note the dilated connecting pieces. Magnification, 20.

Fig. 32 (rabbits 9 and 19).—Dilatation of Bowman's spaces of glomeruli in the central and lateral areas after ninety days' obstruction. One is from a sixty-one day kidney. Magnification, 20.

Fig. 33 (rabbit 16).—Remnants of central collecting tubules from the inner zone of the medulla after twenty-eight days' obstruction. Magnification, 20.



Figures 23 to 33

EXPLANATION OF FIGURES 34 TO 42

Fig. 34 (rabbit 8).—Pigmented distal convolutions from the central area after forty-six days' obstruction. Magnification, 20.

Fig. 35 (rabbit 19).—Remnants of pigmented distal convolutions from the central area after sixty-one days' obstruction. Magnification, 20.

Fig. 36 (rabbit 19).—Blind and flattened collecting tubules to the lateral zone after sixty-one days' obstruction. Magnification, 20.

Fig. 37 (rabbit 13).—Atrophic, blind and flattened collecting tubules to the lateral area after one hundred and fourteen days' obstruction. Magnification, 20.

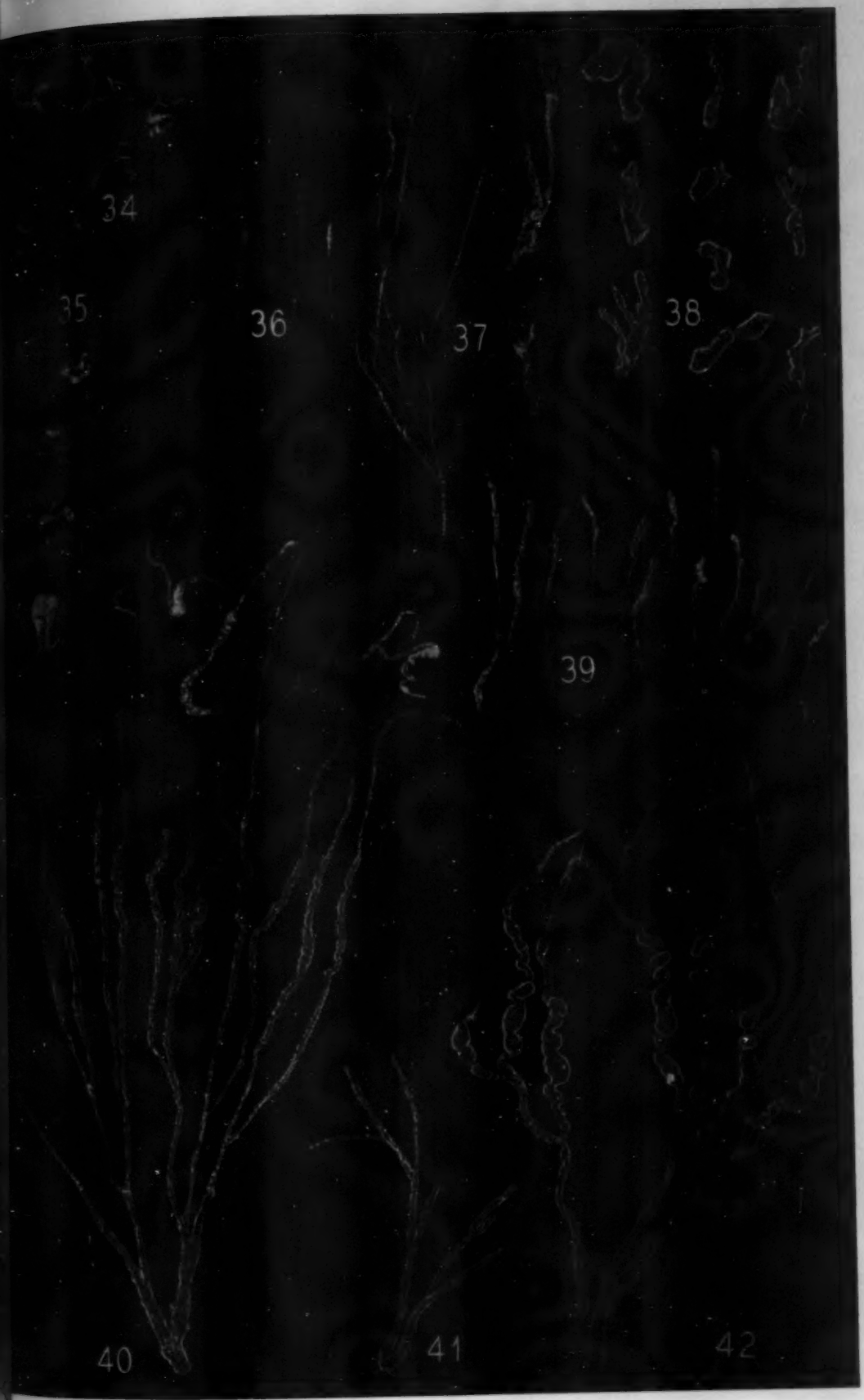
Fig. 38 (rabbit 10).—Blind cystic remnants of collecting tubules from the central portion of the medulla after one hundred and sixty-four days' obstruction. Magnification, 20.

Fig. 39 (rabbit 11).—Remnants of central cortical collecting tubules after two hundred and thirty-one days' obstruction. Magnification, 20.

Fig. 40 (rabbit 17).—Collecting tubules and pigmented distal convolutions from the central portion after thirty-five days' obstruction. Magnification, 20.

Fig. 41 (rabbit 11).—Remnants of branching medullary collecting tubules from the intermediate area after two hundred and thirty-one days' obstruction. Magnification, 20.

Fig. 42 (rabbit 19).—Dilated collecting tubules from the central area after sixty-one days' obstruction. Note the tapering blind ends at the corticomedullary border. Magnification, 20.



Figures 34 to 42

frequency they are cut off from both their collecting tubules and Henle's loops and so lie free, decreased greatly in size and identifiable only by their characteristic contour and pigment content (figs. 34 and 35). In the lateral portion of the organ dissectable distal convoluted tubules are not evident after a few weeks, but in the intermediate area one can find distal convoluted tubules of nearly normal contour in association with better preserved proximal convoluted tubules to one hundred and fourteen days.

Collecting Tubules.—(*a*) Microscopic Sections: After twenty-four hours' obstruction considerable and widespread dilatation of the lumens and compression of the epithelial cells of the collecting tubules are evident. This is increased in the three and six day specimens and generally is more marked in the cortical collecting tubules (fig. 4). Many of the tubules contain coagulated fluid which, as in the distal convoluted tubule, does not have the appearance of casts capable of obstructing the lumen. Those ducts of Bellini when seen in section prior to their previously mentioned fragmentation are lined by epithelium which is not flattened but fairly normal in appearance.

After a week the fractured ends of the larger collecting tubules communicate with the pelvic cavity, and the margin of the disrupted papillary tissue is lined by necrotic material and purulent exudate. In succeeding weeks there follows increasing proliferation of connective tissue about the terminal portions of the collecting tubules, and in the twenty-eight day specimen they are completely surrounded by connective tissue, and the continuity of the pelvic epithelium is reestablished by regeneration of flattened epithelial cells of indifferent character. In the sixty-one day and subsequent specimens the collecting tubules are separated from the pelvis by this proliferating connective tissue, which is covered by an uninterrupted epithelial layer.

At ten days and later there is evident a topographic distribution of change in the collecting tubules, which is seen best in the dissected material. The tubules from the central portions of the cortex are increasingly dilated and shortened, while those from the periphery are stretched and narrowed. At fourteen days there is evident narrowing of the collecting tubules at the corticomedullary boundary, in which region the tubules are later interrupted and when finally a dense hyaline scar appears (fig. 53).

In the ten and twenty-one day specimens a few erythrocytes are found in the central collecting tubules, and in the thirty-six day specimen a slight amount of blood is found in the cortical extremities of some of the central collecting tubules.

In the forty-six day specimen the tubules to the lateral area have largely disappeared, and their remnants are enclosed in connective tissue. However, the capillaries between them persist and are filled with blood.

In the ninety day specimen between groups of glomeruli one finds groups of cystic cortical collecting tubules that can be identified by reference to the dissected material as remnants of the medullary rays.

In the one hundred and sixty-four and the two hundred and thirty-one day specimens the cystic collecting tubules of the central portion of the medulla are enclosed in abundant connective tissue and are composed of cuboid epithelial cells with well defined cytoplasmic borders and round or oval nuclei (fig. 9).

(*b*) Microdissection: In the first days of obstruction the collecting tubules generally become increasingly fragile and less refractive, especially in the outer portion of the medulla and cortex, and they also present a slight increase in external diameter in the region of primary branching, in the inner zone of the medulla. The large terminal collecting tubules are at first unchanged (figs. 15 and 16), but after six days, when a definite general increase in external diameter of the remaining collecting tubules is evident, they are not found save as fragments.

At ten days a well defined topographic distribution of the alteration in the collecting tubules is apparent. Those of the central area are dilated fairly uniformly from the pelvis through the connecting piece and distal convoluted tubules (fig. 20). At fourteen days their caliber is decreased at the corticomedullary border, and in the medulla they present an irregular series of minute indentations due to encroachment of connective tissue. In later stages this is increased until they present definite saccular deformities and pursue a tortuous course through the connective tissue enclosing them.

At twenty-eight days, in the inner portion of the medulla some tubules are found completely interrupted, with the blind ends lying adjacent in the softened collagen of the macerated tissue (figs. 31 and 33). At thirty-six days the central collecting tubules are narrowed in the region of primary branching and in the cortex, with associated greater dilatation and irregularity of outline in the outer zone of the medulla (fig. 40). At forty-six days the number of interrupted central collecting tubules is considerably increased. At sixty-one days the central collecting tubules end blindly at the pelvis and also at the corticomedullary border, but the saccular deformity of the persisting portion in the outer zone of the medulla is increased (fig. 42). The cortical collecting tubules frequently are interrupted, forming fusiform structures, which occasionally show cut-off stubs of their branches.

In the remaining specimens of ninety, one hundred and fourteen, one hundred and sixty-four and two hundred and thirty-one days the central collecting tubules persist as blind structures of varied size and form. At ninety days those of the medullary rays are represented by linear series of minute spherical cysts. At two hundred and thirty-one days a few

minute remnants of the cortical collecting tubules persist; some reach the cortical surface and there, branching, are connected by narrow epithelial remnants to minute blind pigmented distal convoluted tubules (fig. 39). In the central portion of the medulla blind spherical, cylindric or branching structures are enclosed in the abundant connective tissue (fig. 38). The centrally placed tubules are more rounded, while laterally the tubular remnants become increasingly slender, flattened or fusiform (fig. 41).

In contrast, the collecting tubules to the lateral portion of the organ are found at ten days to be greatly stretched and thinned and some virtually obliterated in their midportions (fig. 17). In succeeding weeks increasing numbers of more medially placed collecting tubules become similarly involved. At thirty-six days, in the connective tissue adjacent to the pelvic mucosa are found innumerable minute blind fusiform remnants of these collecting tubules. With advance of the lesion these become more frequently interrupted, smaller, more abundant and flattened, and finally identifiable only by branches in the inner portion of the medulla or cortex (figs. 36 and 37).

The intermediately placed collecting tubules are slightly and uniformly dilated at ten days. In appearance they vary with the distance from the central area, on the one side resembling the deformed shortened central tubules and on the other the more slender fusiform structures of the lateral area. In the midportion of this intermediate area they persist fairly straight and uniform in caliber to one hundred and fourteen days and are not interrupted in their course, being in continuity with their distal convoluted tubules and loops of Henle. The possibility that some of these well preserved collecting tubules in the one hundred and fourteen day specimen communicate with the pelvis is not satisfactorily excluded, and in any event it seems that they must have done so shortly before the end of this period.

INTERSTITIAL ALTERATIONS

In the first few weeks the interstitial tissue is edematous, but this becomes progressively less conspicuous after the third week.

The interstitial tissue is promptly infiltrated by polymorphonuclear leukocytes and round cells, and these are constantly present in the hydronephrotic kidney and, although varying in degree in different specimens, become more prominent with development of the lesion (figs. 4 to 9). Distinct aggregates of round cells are present in the cortex as early as twenty-one days after ureteral ligature. No anywise comparable leukocytic infiltration is ever observed in microscopic sections prepared in the routine manner from the opposite kidney.

An increase in the size of the nuclei of connective tissue cells is generally evident after one day, and after a week a diffuse proliferation

of connective tissue is found. In succeeding weeks this becomes more pronounced with particular localizations. It is prominent about the fragmented remnants of the collecting tubules of the papilla and also in the medulla about the collecting tubules of the central area. Here at ten days compression of the loops of Henle and serial constriction of the collecting tubules are evident, with later production of saccular deformities, linear series of spherical cysts and the bizarre structures of the advanced stages (fig. 38).

Discrete scars are evident early about the larger blood vessels. At fourteen days the central collecting tubules are narrowed at the corticomedullary boundary; at sixty-one days they are interrupted at this point, and later in this region a dense zone of hyalinized collagen is present (fig. 53).

In this study both in the microscopic sections and in the dissected material structural change in the blood vessels appears insignificant. In the early stages there is considerable congestion of the capillaries and veins without topographic localization in the sections examined. The glomerular capillaries are quite uniformly compressed, and their blood content is less than normal.

After ninety days moderate thickening and hyalinization of the smaller arterial branches are observed. Connective tissue proliferation within the capillary tuft is seen first after two hundred and thirty-one days.

The thickening of the arterioles in the cortex (fig. 9) may be due in part to the concentration of glomeruli with associated shortening of the vascular tree.

TOPOGRAPHY OF HYDRONEPHROTIC KIDNEYS

For purposes of illustrating the topographic relations of the altered nephrons and collecting tubules the experiments have been grouped according to their possession of certain common attributes. The outline of a renal section typical of each period has been traced, and camera lucida drawings of actually dissected structures placed within it in the positions noted at the time of dissection. Thus the topographic figures 43 to 54 are not diagrammatic except in the sense that they represent a synthesis of the material from several kidneys.

One and Three Days of Obstruction (figs. 43 and 44).—In this period occur the flattening and splitting of the papilla (fig. 1) which precede separation of the fragmented ducts of Bellini. Otherwise, in this interval there is no localization of change within the organ. However, considerable and fairly widespread dilatation of tubular lumens with compression of epithelial cells rapidly develops, and this is evidenced in the dissected material by increased fragility and translucence

of the nephrons. In addition there is a prompt and readily apparent decrease in the size of the proximal convoluted tubules.

At the end of twenty-four hours' obstruction there is a slight increase in the external diameter of the collecting tubules in the region of the primary branching, and after three days there is a slight general increase in the external diameter of most of the collecting tubules peripheral to this point.

Six, Ten and Fourteen Days of Obstruction (figs. 45 and 46).—In this period there occurs only a slight increase in the volume of pelvic fluid, but with its accumulation there develops obliteration of the papilla with separation of the disrupted extremities of the collecting tubules. There also becomes apparent a topographic distribution of change in the nephrons throughout the kidney, and this structural pattern persists in later stages of the study.

The glomeruli in the lateral portion of the organ become flattened, with their long axes parallel to the capsular surface.

Atrophy of the proximal convoluted tubules increases and becomes especially marked in the central and lateral portions of the organ, with persistence of less atrophic units in the intermediate area.

The long loops of Henle, particularly those of the central area, extend into the marginal necrosis of the fragmented papilla, and other Henle's loops are found modified in accordance with structural alterations in the adjacent collecting tubules.

The central collecting tubules become increasingly dilated and translucent, and this change extends commonly to the loops of Henle. The collecting tubules to the lateral area are stretched and narrowed in their midportions, and some are interrupted. The collecting tubules of the intermediate area are dilated slightly and quite uniformly from the pelvis to their distal convoluted tubules.

Twenty-One and Twenty-Eight Days of Obstruction (figs. 47 and 48).—During this interval the volume of pelvic fluid is about doubled and has become blood tinged. The continuity of the pelvic and tubular epithelium is reestablished, and fibrosis within the parenchyma is conspicuous, especially in the central portion of the medulla and cortex.

Atrophy of proximal convoluted tubules is increased, and dissectable proximal convoluted tubules are rarely encountered save in the intermediate area.

The loops of Henle in the central portion of the medulla are increasingly compressed by the dilatation of the adjacent collecting tubules and by the proliferating connective tissue. Those long loops of the lateral area are stretched, and no particular shortening of the loops of Henle is apparent.

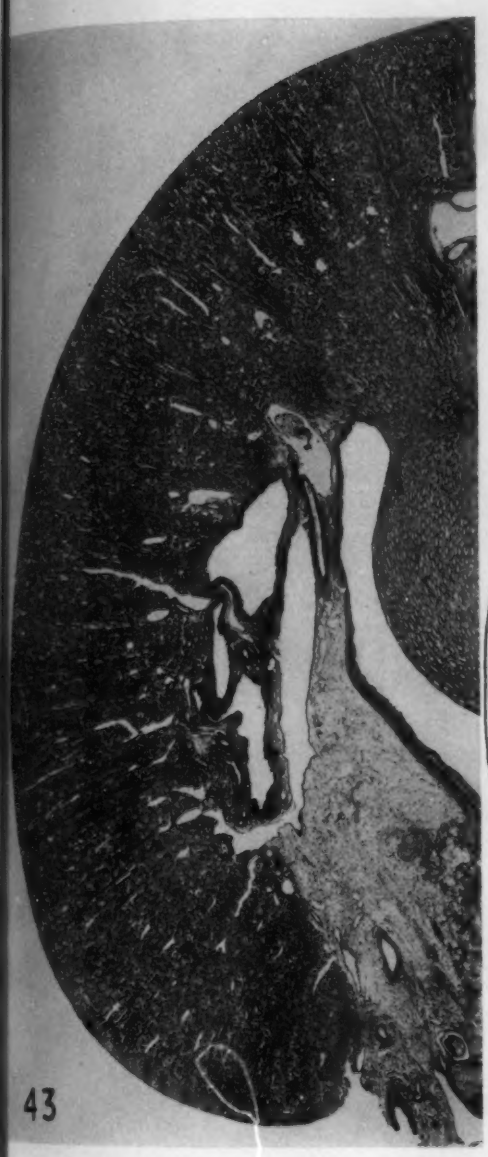


Fig. 43 (rabbit 6).—One day's obstruction. Magnification, 6.

Fig. 44.—Camera lucida drawings of actually dissected structures from kidneys obstructed for intervals of one and three days placed in the position noted at the time of dissection. During this interval there is no localization of change within the nephrons.

Fig. 45 (rabbit 14).—Ten days' obstruction. Magnification, 6.

Fig. 46.—Six, ten and fourteen days' obstruction. Topographic distribution of change is evident after ten days' obstruction. This is emphasized in the increased atrophy of the proximal convoluted tubules in the central and lateral areas and in the shortening and stretching of their respective collecting tubules.

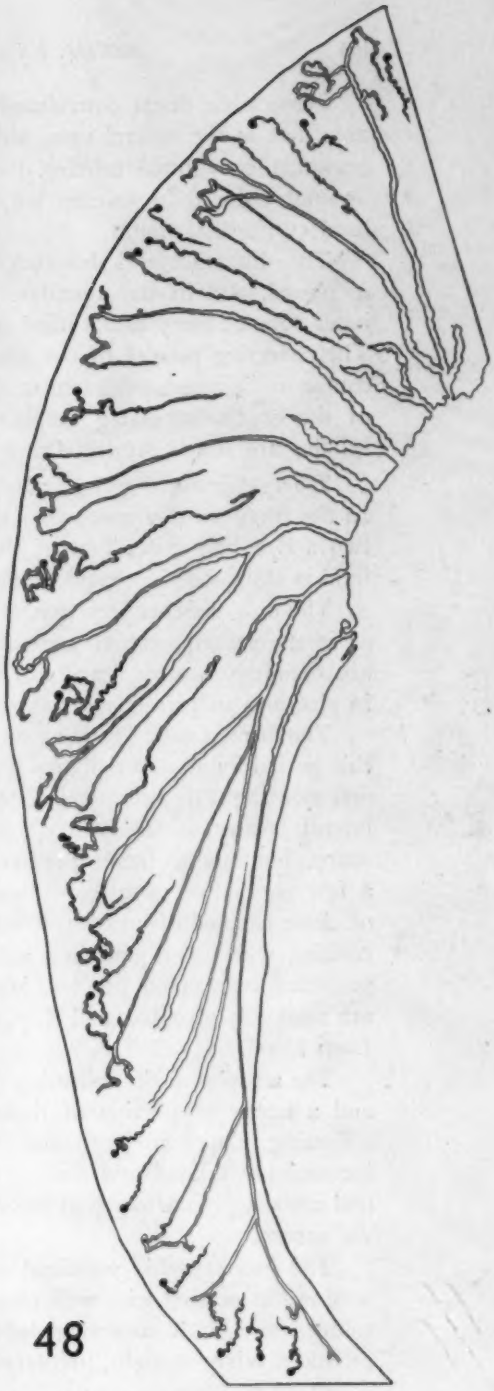


Fig. 47 (rabbit 16).—Twenty-eight days' obstruction. Magnification, 6.

Fig. 48.—Twenty-one and twenty-eight days' obstruction. Few dissectable proximal convoluted tubules are found save in the intermediate area.

Dissectable distal convoluted tubules are very rare in the lateral area, but in the central area, although there is marked atrophy of the proximal convoluted tubules, the associated distal convoluted tubules are not reduced in volume but, on the contrary, occasionally present local cystic dilatation.

The dilatation and deformity of the central collecting tubules are increased, and in the abundantly developing interstitial tissue in the inner zone of the medulla blind remnants of these tubules are observed. The collecting tubules of the intermediate area pursue a fairly straight course and present moderate uniform dilatation. In the lateral portion of the organ increasing numbers of more medially placed collecting tubules are found stretched and interrupted.

Thirty-Six and Forty-Six Days of Obstruction (figs. 49 and 50).—In the forty-six day specimen (fig. 2) the volume of the pelvic fluid is two and a half times that of the twenty-eight day specimen, and the fluid is composed in considerable part of fresh blood.

The only dissectable proximal convoluted tubules in these specimens, as in the subsequent ones, are found in the intermediate area. These are definitely atrophic, and adjacent tubules in this area vary greatly in size and in refractive qualities.

The topographic distribution of change is further emphasized in this period by an accumulation of blood in the distal convoluted tubules and to a slight degree in the collecting tubules of the central area. The lateral border of the area of pigmented distal convoluted tubules is sharp, but among better preserved elements of the intermediate area a few pigmented distal convoluted tubules are found. Several groups of these isolated distal convoluted tubules are traced to single collecting tubules, which also contain a slight amount of blood. The associated proximal convoluted tubules, identified by their relation to glomeruli, are markedly atrophic, and they, together with their glomeruli, are free from blood.

The atrophy of the collecting tubules to the lateral area has increased, and a larger proportion of these tubules is interrupted. The central collecting tubules are narrowed in the region of primary branching, are increasingly dilated and constricted in the outer zone of the medulla and are sharply narrowed at the corticomedullary boundary and through the cortex.

The less atrophic proximal convoluted tubules in the intermediate area are associated with well preserved, nonpigmented distal convoluted tubules and with collecting tubules which, although slightly dilated, pursue a fairly straight, uninterrupted course.

Sixty-One, Ninety and One Hundred and Fourteen Days of Obstruction (figs. 51 and 52).—In this interval the volume of pelvic fluid has

Fig. 49 (rabbit 17).—Thirty-six days' obstruction. Magnification, 6.

Fig. 50.—Thirty-six and forty-six days' obstruction. The topographic distribution of change is emphasized by the accumulation of blood in the distal convoluted tubules of the central area.

decreased to about 25 per cent of that found in the forty-six day specimen and has become less turbid and is free from obvious blood.

In the central and lateral areas there is considerable cystic dilatation of some glomeruli in the inner portion of the cortex in direct relation to the larger arcuate vessels.

Persistent remnants of pigmented distal convoluted tubules occur in a distribution similar to that noted previously. In this area and in the lateral area proximal convoluted tubules are identifiable occasionally as minute twigs on glomeruli opposite the vessel pole.

The central collecting tubules are interrupted at the corticomedullary border and are interrupted in the cortex repeatedly with the formation of slender fusiform structures and linear series of minute spherical cysts. The collecting tubules of the lateral area exist as blind flattened structures, identifiable with certainty only in the regions of branching in the inner portion of the medulla and cortex.

The collecting tubules are separated from the pelvis by connective tissue, and the pelvis is lined by intact epithelium.

The last dissectable nephrons are found in the intermediate area of the one hundred and fourteen day specimen. Their collecting tubules, distal convoluted tubules and Henle's loops, in so far as the latter could be followed, are of relatively normal contour, and their proximal convoluted tubules are slightly larger than those found in the corresponding position in the sixty-one day specimen. These elements of the one hundred and fourteen day specimen contrast with the extremely atrophic remnants of the ninety day specimen, in which no intact nephrons are found.

One Hundred and Sixty-One and Two Hundred and Thirty-One Days of Obstruction (figs. 53 and 54).—During this period the volume of pelvic fluid is reduced further to 50 per cent of that at one hundred and fourteen days of obstruction, and in some dimensions the hydronephrotic kidney is reduced below the normal. The pelvic fluid, however, even at this stage is not quite clear but remains slightly turbid and yellowish.

The glomeruli are remarkably close together, being separated only by collagen fibrils and an occasional tubular remnant. Proximal convoluted tubules are not identifiable. Occasional blind remnants of pigmented distal convoluted tubules are found in the central portion of the cortex.

The blind, cystic medullary collecting tubules are of variable size and contour, depending on their branching and their distance from the central area. With progression laterally from the central part of the medulla they become increasingly more slender and shorter until they blend with the exceedingly atrophic, undissectable remnants of the collecting tubules to the more lateral portions of the organ.

Fig. 51 (rabbit 19).—Sixty-one days' obstruction. Magnification, 6.

Fig. 52.—Sixty-one, ninety and one hundred and fourteen days' obstruction. Cystic dilatation of the glomeruli is noted in the central and lateral areas. Pigmented remnants of distal convoluted tubules persist in the central area. The last dissectable nephrons are found in the intermediate area in the one hundred and fourteen day specimen.

Fig. 53 (rabbit 11).—Two hundred and thirty-one days' obstruction. Magnification, 6.

Fig. 54.—One hundred and sixty-one and two hundred and thirty-one days' obstruction. The cortex is composed of concentrated glomeruli separated by collagen fibrils and minute unidentifiable tubular remnants. The collecting tubules of the medulla vary in size and contour, in their branching and in their distance from the central area, blending laterally with undissectable tubular remnants.

COMMENT

For years the literature has contained Cohnheim's [5] authoritative description of typical hydronephrosis produced by sudden, permanent experimental occlusion of the ureters of rabbits and dogs. Although he contrasted the "moderate" hydronephrosis developing after sudden, persistent ureteral occlusion with the really voluminous hydronephrosis found in animals with slowly developing, partial or intermittent obstruction, his observations offer no basis for confusion and are in accord with common experience. He described the yielding of the pelvis to the accumulating fluid and the setting in of absorption of the fluid. He believed it unnecessary to have recourse to the possibility of continued secretion by the pelvic mucosa to explain the increase in volume of the pelvic fluid because he found that the kidney had by no means ceased to secrete. His experimental results were amply confirmed several decades ago by Guyon and Albarran,[6] Fabian,[7] Ponfick [1] and Suzuki.[8]

Nevertheless, suggestions persist that sudden complete ureteral obstruction is not followed by accumulation of pelvic fluid, and from this erroneous idea, and not from direct anatomic evidence, prompt parenchymal atrophy has been inferred to account for the supposed lack of accumulating fluid. The lineage of this concept of "primary atrophy" perhaps may be traced through the early work of Lindemann [9] and others, but, as Papin [10] observed, the exceedingly infrequent experimental examples of the so-called primary atrophy were generally recognized at the time as exceptional.

Misquotation of Cohnheim [5] has sustained this notion of "primary atrophy." Such misinterpretation is to be found in the papers of Albarran,[11] Lindemann [9] and Scott [12] and more recently in the work of Suter.[13]

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5. Cohnheim, J.: Vorlesungen über allgemeine Pathologie, Berlin, A. Hirschwald, 1880.

6. Guyon, F., and Albarran, J.: Arch. de méd. expér. et d'anat. path. **2**:181, 1890.

7. Fabian, E.: Pathologie und pathologische Anatomie, in Born, G., and Flügge, K.: Bibliotheca medica, Cassel, T. G. Fischer & Co., 1904, vol. 18, p. 1.

8. Suzuki, T.: Zur Morphologie der Nierensekretion unter physiologischen und pathologischen Bedingungen, Jena, Gustav Fischer, 1912.

9. Lindemann, W.: Ztschr. f. klin. Med. **34**:299, 1898.

10. Papin, E.: Les hydronéphroses, Paris, Gaston Doin, 1930.

11. Albarran, J.: Exposé des travaux scientifiques de J. Albarran, Paris, Masson & Cie, 1906.

12. Scott, G. D.: Surg., Gynec. & Obst. **15**:296, 1912.

13. Suter, F., in von Bergmann, G., and Staehelin, R.: Handbuch der inneren Medizin, Berlin, Julius Springer, 1931, vol. 6, pt. 2.

It is to be found also in the current edition of a leading American textbook of pathology:[14] "Cohnheim made the statement that only partial or intermittent obstruction is followed by great distension of the pelvis, while complete obstruction results in cessation of the flow of urine and atrophy of the kidney."

Although this concept of "primary atrophy" had no acceptable demonstrated basis in reality, and although it was adequately refuted more than three decades ago, the extreme expression of a converse conception is found in Hinman's[15] opinion that the "pyelovenous backflow" from the pelvis of an actively functioning hydronephrotic kidney permits maintenance of a "fresh water lake continuously renewed by active secretion above."

Ponfick's[1] studies led him to conclude that the rate of accumulation of pelvic fluid and the rate of atrophy of the parenchyma are in agreement; that reciprocal relations exist between the mass of the pelvic fluid and the mass of the parenchymal elements. For this reason it appeared to him that in later stages and in higher degrees of pelvic distention an ever increasing proportion of the accumulating fluid must flow from the pelvic mucosa. In the present study the rate of accumulation of pelvic fluid and the rate of atrophy of the parenchyma are found to be in accord with the corresponding data of Ponfick, and, although the pelvic content obviously is not "stagnant," it at no time resembles a "fresh water lake."

It is commonly acknowledged that the pelvic fluid quickly loses the character of urine, and it must therefore be granted that the organ has ceased to function as a kidney. Although for a considerable period after ureteral ligation the highly vascular renal parenchyma seems an obvious source of pelvic fluid, and although the analogy drawn between hydronephrosis and hydrops of the gallbladder is an ancient one, no assurance has been offered thus far as to the validity of the varying opinions as to the source of the fluid. Thus the accumulating pelvic fluid toward which, as Ponfick stressed, attention so largely has been directed by the prejudicial term "hydronephrosis" loses its immediate importance as a direct measure of the parenchymal secretory activity and structural change.

The present study has shown that the rate of accumulation of pelvic fluid rises sharply between four and eight weeks after ligation of the ureter, and this change in rate is due largely to bleeding into the pelvis. Inasmuch as no source of bleeding has been found within the parenchyma

14. MacCallum, W. G.: Text-Book of Pathology, ed. 6, Philadelphia, W. B. Saunders Company, 1938, p. 438.

15. Hinman, F., in Nelson Loose-Leaf Living Surgery, New York, Thomas Nelson & Sons, 1937, vol. 6, p. 545.

even on histologic examination, it is assumed that the hemorrhage may be from peripelvic veins. This rapidly developing hemorrhagic character and sharp increase in volume of the pelvic fluid within a limited interval of time have not heretofore been recognized. Although these features were completely reproducible in a series of 6 additional animals used for verification of these points, and although in this aspect of the problem as in others there is, in general, progressive agreement in the course of the process in the animals studied, and although in the literature there is indirect confirmation, one can conceive of circumstances which might lead to nonappearance of the bleeding, such as an unusual distribution or character of peripelvic vessels or a failure of pelvic fluid to accumulate to the critical volume required to produce the striking circulatory disturbance which in this series has constantly developed.

In the thirty-six day specimen of his series Ponfick [1] did not describe an especially hemorrhagic fluid but noted rust brown layers of erythrocytes on parts of the pelvic mucosa and commented on their relative abundance in the pelvic fluid in contrast to their isolated appearance in the parenchyma. He had no animals with ureters obstructed for intervals between thirty-six and one hundred and twenty days, and Fabian,[7] although he mentioned ligation of the ureters of 125 animals, listed none as examined in the interval between twenty-five and fifty days among the 17 for which individual protocols were offered. However, Ponfick [1] and Suzuki [8] offered undeniable corroborative evidence, shortly to be mentioned.

The early splitting of the papilla with disruption of the ducts of Bellini and later separation of the torn ends of the collecting tubules due to the accumulation of pelvic fluid is a significant injury with respect to both the regional parenchymal changes and the fate of the organ. The unipapillary kidney of the rabbit may be expected to differ somewhat from the multipapillary kidneys of other animals in this feature, and difference in the degree of trauma and in the progress of repair may be significant factors in determining the somewhat variable course this type of experimental hydronephrosis is known to follow in these animals (Fabian [7]). It seems likely that these factors may be responsible for the irregularities in the atrophy and in the preservation of nephrons that are so striking in the intermediate area in the ninety and one hundred and fourteen day specimens of this study.

For a variable period in the early weeks of the obstruction the surface of the fragmented papilla is necrotic and is covered by a moderately abundant purulent exudate. This "necrosis" of the papilla, described by Helmholz and Field [16] and assumed by them to be due to "anemia," is produced by the physical trauma that results from increas-

16. Helmholz, H. F., and Field, R. S.: J. Urol. **15**:409, 1926.

ing pelvic distention. Kelly and Burnam [17] suggested that compression of the ducts of Bellini due to papillary flattening creates a valvelike structure of the papilla, and Hinman and Lee-Brown [18] compared this "valve" to the ureterovesical valve and discovered that the collecting tubules become collapsed and even more "valve-like" as hydronephrosis progresses. On the contrary, as has been noted, the papilla of the rabbit kidney is torn apart by the pressure of the accumulating fluid.

Reports in the literature repeatedly speak of glomerular "dilatation" as a consequence of obstruction This has been found to be rare and to occur, as Zurhelle [19] observed, only in certain glomeruli in later stages of obstruction. No significant increase in external diameter is found on measurement of glomeruli in tissue sections, and such is not evident, save as mentioned, in the macerated material, in which recognition of variation in glomerular form and volume is readily possible.

The long recognized lack of correlation between glomerular and "tubular" atrophy in hydronephrosis is evident at an early period in the dissected material. This disproportion is most obvious between glomeruli and the proximal convoluted tubules.

In the literature one encounters numerous descriptions of curious structural changes that have not been found in the present study. Among them might be mentioned the fusion between the capillary loops and the parietal layer of Bowman's capsule and the disappearance of the capsular space noted by Gruber,[20] the glomeruli lying loose in their capsules described by Orth [21] and the constant form of a flattened hexagon assumed by Bowman's capsule described by Ponfick.[1]

In past descriptions of the effect of hydronephrosis on the renal parenchyma there is almost universal uncertain usage of the term "dilatation." No distinction is made between increase in caliber of lumen and increase in external diameter of the tubule. The reader is therefore left uncertain as to whether any increase in the size of affected nephrons has occurred, for a tubule with a dilated lumen may or may not be a "distended" tubule.

Hypertrophy of the proximal convoluted tubules was never observed, in contrast to the finding of Hinman,[15] nor was the external diameter of these tubules ever found to be greater than normal, in spite of frequent mention in the literature of their "dilatation." Evidence of injury of their epithelial cells is to be found after twenty-four hours' obstruc-

17. Kelly, H. A., and Burnam, C. F.: Diseases of the Kidneys, Ureters and Bladder, New York, D. Appleton and Company, 1914.

18. Hinman, F., and Lee-Brown, R. K.: J. A. M. A. **82**:607, 1924.

19. Zurhelle, E. F.: Frankfurt. Ztschr. f. Path. **10**:42, 1912.

20. Gruber, G. B., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1934, vol. 6, pt. 2.

21. Orth, J.: Virchows Arch. f. path. Anat. **202**:266, 1910.

tion, and a moderate, irregularly distributed increase in caliber of their lumens is also to be seen at this time. Early degenerative change in the epithelial cells of the proximal convoluted tubules is mentioned frequently in the literature.[22]

Suzuki,[8] even using intravenous injections of carmine as a means of localization and so gaining an especial appreciation of the effect of obstruction on the proximal convoluted tubules, gained only a suspicion of the rapidity and degree of their atrophy. Further, his conclusion is weakened by his frank admission that his effort to distinguish between different types of tubules in later stages of the lesion was carried to that fine point where he no longer was certain that he was telling them apart.

The most striking change in the proximal convoluted tubule is therefore their prompt and marked reduction in volume. This, indeed, is noted in the first week of obstruction but can be appreciated only in the dissected material, in which many atrophic proximal convoluted tubules can be observed at a glance and readily compared with the normal.

The most obvious condition associated with the atrophy of the proximal convoluted tubule in time and place, which usually is the essence of the establishment of a "causal relation," is urinary obstruction. The importance of this condition as the cause of parenchymal change is supported further by the more rapid atrophy occurring in the areas where secondary blockage of the tubules can be demonstrated to exist within the nephrons—i. e., in the central and lateral portions of the organ, where after twenty-eight days dissectable proximal convoluted tubules are no longer to be found—and by the fact that in the intermediate area where such secondary blockage is not to be demonstrated the least atrophic nephrons, the ones of more nearly normal contour, are to be found. Obviously, innumerable secondary causal relations develop, but thus far no one has been able to extricate himself successfully from efforts to evaluate the relative importance of, for example, the altered blood supply and interference with function and the distribution of pressure as factors in determining the genesis of hydronephrosis.

Thus the concept of atrophy of the kidney developing promptly after obstruction of urinary outflow appears again but established on objective anatomic evidence and including a regional distribution within the nephron and within the organ. The concept's approach to reality becomes topographic and quantitative and a function of time elapsed.

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22. (*a*) Heidenhain, R.: Arch. f. d. ges. Physiol. **9**:10, 1874. (*b*) Joelson, F. J. E.; Beck, C. S., and Moritz, A. R.: Arch. Surg. **19**:673, 1929. (*c*) Boetzel, E.: Beitr. z. path. Anat. u. z. allg. Path. **57**:294, 1913. (*d*) Ponfick.[1]

Inasmuch as the proximal convoluted tubules form the chief bulk of the organ, their rapid atrophy is a factor of the greatest importance in facilitating the accumulation of pelvic fluid and in determining the architecture of the hydronephrotic kidney.

The only localized change found with any regularity within individual proximal convoluted tubules corresponds with a localization found by Suzuki,[8] who noted that after nine days of obstruction, although the epithelial cells of the initial portion of the proximal convoluted tubule were almost as strongly stained by the previously injected carmine as those of the opposite side, the terminal portion of the obstructed proximal convoluted tubule was poorly stained.

Henle's loops maintain their identity through a considerable interval, being shortened or stretched only in proportion to the change occurring in the collecting tubules between which they lie embedded in connective tissue fibrils. They never become distended as do the distal convoluted tubules and collecting tubules, they are not preserved as are the glomeruli, and they show no rapid decrease in size comparable to that of the proximal convoluted tubule, from which they are continually distinguishable (as long as the proximal convoluted tubule can be dissected) by a variable but definite persistence of the original sharp transition.

The loops in the central and lateral areas become thin and delicate more rapidly than those in the intermediate area and with the increase of diffuse interstitial fibrosis are almost impossible to trace after thirty-five days. Their lumens variably increase in caliber, but no increase in external diameter is observed even proximal to obvious obstruction due to connective tissue or to dilated collecting tubules in the central region of the medulla. This interference with the loops of Henle in the central portion of the medulla, along with the tearing of the long loops involved in the disruption of the papilla, obviously provides a secondary interruption in the nephrons and involves chiefly those of the central portion of the parenchyma.

Suzuki [8] noted that the ascending limb of Henle is "dilated" and includes this together with the distal convoluted tubules in his dilated system of tubules. This again emphasizes the loose application of the term "dilatation." Contradictions in its use are too numerous and too unimportant to mention further save for the interesting contrast provided by Scott,[12] who described "dilatation" beginning in the straight tubules and proceeding through the proximal convoluted tubules to the glomeruli, and Johnson,[3] who described "dilatation" primary in the glomeruli with progressive extension through the tubular system to the pelvis. In the present study the only "distended" tubules encountered have been the distal convoluted tubules and the collecting tubules of certain portions of the kidney.

In the aforementioned study by microdissection [3] there are described a shortening and drawing up of the long loops of Henle with the consequent disappearance of the loop so that the glomerulus comes to lie directly attached to the collecting tubule. No dissections are shown to support this conclusion, reference being made for its illustration to a diagram which represents obviously a subjective interpretation rather than a reality of observation. Such was not found in the material of the present study, but appearances that conceivably might give rise to such an interpretation were provided roughly by arteries with glomeruli attached or by collecting tubules and distal convoluted tubules with glomeruli attached. The glomeruli normally are attached to their distal convoluted tubules by fibrous strands of greater tensile strength than is possessed by the delicate neck of the proximal convoluted tubule and so may remain attached to the distal convoluted tubule on dissection of the macerated tissue.

In contrast to the absence of distention in the proximal convoluted tubules and loops of Henle at any stage of the hydronephrotic alteration, an increase in the external diameter of many of the distal convoluted tubules and collecting tubules develops early and not infrequently reaches for a time cystic proportions. In the kidneys of a patient with Bright's disease in the terminal stage Oliver [23] found remarkable dilatation of Henle's loops and of proximal convoluted tubules as a result of obstruction due to filling of dilated distal convoluted tubules and collecting tubules with solid debris. There exists an obvious difference in the gradual development of occlusion of the individual nephrons of the kidney in Bright's disease and the sudden interference with ureteral flow in experimental hydronephrosis with the complicating factor of pelvic distention, which doubtless tends to prevent spread of intratubular pressure throughout the kidney.

On the other hand, this difference in behavior contrasts with the striking similarity of the structural form of the dilated and deformed collecting tubules found in the central portion of the medulla in the later stages of hydronephrosis (fig. 42) and the pattern of collecting tubules isolated from kidneys of patients with Bright's disease in the terminal stage by Oliver.[24]

The value of the method of microdissection in revealing the arrangement of nephrons into an organ is well illustrated in the hydronephrotic rabbit kidney because of the relative simplicity of the general topographic pattern that is reduplicated in the successive stages of the experimental lesion. Despite this simplicity, efforts to localize such change within the kidney by a study of microscopic sections have been largely unproduc-

23. Oliver,[2d] p. 90, plates XVIII-XXI.
24. Oliver,[2d] plate VI, fig. 44.

tive. In the literature [25] one frequently encounters the term "group atrophy" of parenchymal elements without intimation as to the constitution of the "groups." The only possibilities suggesting themselves as explaining the use of this term is that repeated cuts across a single proximal convoluted tubule or that the previously mentioned cystic tubules of the medullary rays may have been observed.

Suzuki [8] found that the best preserved tubules were in the "medial-sagittal" section of the kidney and that the rate of atrophy was directly related to the distance from the papilla. The more rapid atrophy of the lateral portion of the kidney has been generally recognized, and Suzuki attributed it to the fact that the collecting tubules adjacent to the pelvis are more readily compressed by the direct pressure of the accumulating pelvic fluid. However this may be, as microdissection shows, these collecting tubules are stretched and interrupted early, and this is the chief finding related to the more prompt atrophy of the lateral area of the kidney. The importance of casts as causes of secondary obstruction within the kidney, emphasized by Ponfick,[1] is not borne out by an examination of the tubules in their actual continuity in microdissection. Detritus was rarely seen plugging tubules and then was found in tubules already interrupted.

With accumulation of pelvic fluid and advance of the parenchymal lesion there is progressive narrowing of the intermediate zone of the kidney, which undergoes minimal disturbance in general arrangement. This smaller distortion together with the slighter involvement of the collecting tubules and Henle's loops in the fragmentation of the papilla and the lesser proliferation of the interstitial tissue is associated with the fact that some of the nephrons in this zone maintain their identity as morphologic units for as long as one hundred and fourteen days. It is to be observed that after thirty-five days' obstruction some of the proximal convoluted tubules in this area possess mitochondria of nearly normal arrangement.

The distance between the pelvis and the cortical surface in the central area is quickly reduced with the accumulation of pelvic fluid, and thus there develops a considerable difference in length of collecting tubules between the central and the intermediate zones. The central collecting tubules also promptly become distended, and this distention increases in certain regions to a considerable degree. Contrary to the findings of Suzuki,[8] the atrophy of the proximal convoluted tubules in the central area is nearly as rapid as is that in the lateral area. This is associated wth the greater involvement of the long Henle's loops in the fragmentation of the papilla and the compression of these loops by the promptly distended

25. (*a*) Hinman, F.: Surg., Gynec. & Obst. **58**:356, 1934. (*b*) Hinman, F., and Morrison, D. M.: Surg., Gynec. & Obst. **42**:209, 1926. (*c*) Hinman.[15] (*d*) Joelson.[22b]

collecting tubules and by the early and marked proliferation of connective tissue in this area. Thus, although there is rapid atrophy of the proximal convoluted tubules in both the lateral and the central portion of the kidney, the collecting tubules in the former area are stretched and interrupted while in the latter portion of the kidney they are shortened and dilated. The pressure within the central collecting tubules is evidently sufficient to maintain them together with their distal convoluted tubules as patent tubes for a considerable period after their respective proximal convoluted tubules and Henle's loops are markedly atrophied. That for a time considerable fluid flows from the pelvis through these short, dilated central collecting tubules to be absorbed in the distal convoluted tubules will be evident from the following considerations.

After thirty-six days' obstruction the distal convoluted tubules in the central area are dilated and filled with blood. The sudden increase in size and the close packing of the erythrocytes indicate that within a short period there must have been a considerable backflow of blood and absorption of fluid from the distal convoluted tubules.

The source and peculiar distribution of this blood puzzled Ponfick,[1] who noted it first in his thirty-six day specimen. He sensed that it might yield important information about the genesis of hydronephrosis but, being unable to identify the tubules in which it occurred, saw only that in the progressive changes occurring in the blood cells there existed a guide to the duration of the lesion.

Suzuki[8] found blood with identical distribution in his forty-one day specimen and also in his later specimens and noted the development of stainable iron within it. Suzuki likewise failed to localize the blood within the distal convoluted tubules—an identification impossible in the microscopic sections that is obvious in the dissected material. Suzuki thought that the blood might come from the glomerulus but was dissatisfied with this conjecture and offered as an alternative hypothesis that it might come from local rupture of vessels into adjacent tubules. Such an idea, unsupported as it is by direct evidence, need not be maintained in face of the previously described unique distribution of this blood. Furthermore, this phenomenon is coincident with massive bleeding into the pelvis of the kidney from no identifiable parenchymal source.

Backflow is manifestly impossible in the interrupted collecting tubules of the lateral area, and while it occurs in some of the nephrons of the intermediate area which have exceedingly atrophic proximal convoluted tubules, it does not occur into those distal convoluted tubules which are associated with the least atrophic proximal convoluted tubules of this area. The relative morphologic integrity of these nephrons suggests that in many of this numerically insignificant group there may be a flow directed toward the pelvis. As an important factor in hindering

blackflow into the collecting tubules of the intermediate area their greater length also must be considered. Blood was not found in collecting tubules unless it had progressed to and accumulated in the distal convoluted tubules.

The dilatation and deformity of the distal convoluted tubules and the filling of their loops by solid material are similar to the finding of Oliver in the kidneys of the patient with Bright's disease [26] and may be considered as additional evidence that the distal convoluted tubules are the normal sites of absorption of fluid and are points where debris may be solidified into obstructing solid masses.

No changes have been found in the vessels in tissue sections other than fairly uniform anemia of the glomerular capillaries and moderate to marked congestion of the larger veins in early stages, and no changes have been found in the dissection which permit speculation as to the efficiency of these vessels. To determine the minute volume of blood flowing through them by looking at minor changes in the artefacts of microscopic sections would seem impossible. On such a basis there has been considerable speculation concerning the obvious circulatory disturbance occurring in the hydronephrotic kidney. In perfusion experiments Ghoreyeb [27] found evidence of retarded flow through the obstructed kidney, initially only in the presence of the accumulated pelvic fluid, but after a week, on removal of the fluid. He attributed the difference in retardation of flow to proliferative changes in the interstitial tissue.

By another approach, the injection of barium sulfate gelatin and the celluloid corrosion method, Hinman and Morison [25b] demonstrated an altered arrangement of the larger vessels and a decrease in number of the finer arterial branches of the hydronephrotic rabbit kidney after seventy days of complete ureteral obstruction. His conclusion that "atrophy" of the finer arterial branches had occurred and his further conclusion that parenchyma persists which is in direct relationship to the major branches of the renal artery are not borne out by the results of the present histologic study or by microdissection.

In the present study the persistent parenchyma was found to be very definitely related topographically to those distortions of the organ which permitted maintenance of the integrity of its nephrons exclusive of any possible effect of blood supply, and further this area of preserved nephrons exists in the material studied in a zone at right angles to the course of the larger renal vessels.

That the examination of the vessels of this material was not neglected may be indicated by the fact that they were studied with sufficient intensity to support with objective evidence a new general concept of the organization of the media of the distributing arteries.[28]

26. Oliver,[2d] plate V, figs. 35 and 37 and text figures 33 and 53.
27. Ghoreyeb, A. A.: J. Exper. Med. **20**:191, 1914.
28. Strong, K. C.: Anat. Rec. **72**:151, 1938.

The edema occurring in the early weeks of obstruction was described and discussed by Ponfick [1] and others. Infiltration of the obstructed kidney by polymorphonuclear leukocytes and round cells is prompt, marked and persistent, and, contrary to the experience of Johnson,[29] no spontaneous nephritis was encountered in the right kidneys in any of the experiments of this study.

The early extensive acute inflammation and marked fibroblastic proliferation in the peripelvic tissue have been especially described by Helmholz and Field.[16]

Changes in the interstitial tissue as well as epithelial injury are manifest after twenty-four hours' obstruction, and the degradation of the parenchymal elements and the connective tissue proliferation are apparently simultaneous and interdependent. Thus, unfortunately, no light is shed on the classic dispute between Ziegler and Weigert, but it is evident that the inflammatory reaction leads to fibrosis and distortion of the parenchyma in the development of hydronephrosis as it does in other forms of chronic renal disease. The ability of these parenchymal elements to resist molding and loss of continuity due to the developing inflammatory reaction, if opportunity for function is provided, is demonstrated by the experiments of Boetzel,[22c] Rautenberg,[30] Kawasoye,[31] Johnson [29] and Joelson, Beck and Moritz.[22b] They showed that after several weeks of complete obstruction the previously hydronephrotic kidney when offered a chance for physiologic activity is capable of an amazing anatomic restitution and when given the stimulus can become capable of carrying the total load of renal function.

Prof. Jean R. Oliver gave help and advice during the progress of this work. Elizabeth Dunn prepared the microscopic sections; Elizabeth Cuzzort, the drawings of dissected specimens (figs. 10 to 42); Muriel MacDowell, the illustrations of the topographic relations, and Steven Montes, the photographs.

29. Johnson, R. A.: J. Exper. Med. **28**:193, 1918.
30. Rautenberg, E.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **16**:431, 1906.
31. Kawasoye, M.: Ztschr. f. gynäk. Urol. **3**:172, 1911-1912.

Case Reports

RETICULOENDOTHELIOSIS WITH LIPOID STORAGE

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The remarkable activity of the reticuloendothelial system in disease results in a large variety of anatomic changes. These disorders are usually classified as inflammatory hyperplasia, neoplasia or abnormal lipoid storage. Gaucher's disease, Hand-Schüller-Christian disease and Niemann-Pick disease were formerly considered disturbances of lipoid metabolism but are now generally regarded as primary diseases of the reticuloendothelial system. For a discussion of these diseases, the reader is referred to the papers of Foot and Olcott,[1] Ritchie and Meyer,[2] Chevrel[3] and Epstein.[4] We are indebted principally to Epstein[4] for the identification of the lipoids in these diseases. In Gaucher's disease one finds the storage of a cerebroside, kerasin, in the spleen, bone marrow and lymph nodes. In Hand-Schüller-Christian disease the xanthomas throughout the body contain cholesterol. In Niemann-Pick disease there is widespread storage of phospholipid.

This report concerns a case of generalized hyperplasia of the reticuloendothelial system associated with storage of a lipoid, cholesterol. It presents certain clinical and pathologic features that are not typical of either reticuloendotheliosis or Hand-Schüller-Christian disease. Dr. E. T. Bell, of the University of Minnesota, assisted in the preparation of the report.

REPORT OF A CASE

A 54 year old white man was well until the spring of 1938, when he noticed a gradual onset of weakness and loss of weight. In April 1938 bronchopneumonia developed, and the patient was admitted to Mount Washington Sanatorium. There was no clinical evidence of pulmonary tuberculosis, and the sputum was negative for acid-fast bacilli. The red cell count at this time was 1,200,000, with hemoglobin 25 per cent (Tallqvist). The patient recovered from the respiratory infection, but the severe anemia persisted in spite of intensive liver and iron therapy. During the summer of 1938, after the extraction of a tooth, he bled profusely. He was admitted to Luther Hospital on September 16 because of severe anemia. On admission his temperature was 98.4 F. He was undernourished and very pale. The general physical examination gave negative results except for great enlargement of the spleen. His red cell count was 1,520,000 and the hemoglobin content was 4.2 Gm. (Sahli). The color index was 0.91. The total leukocyte count

From the Luther Hospital, Midelfart Clinic and Mount Washington Sanatorium.

1. Foot, N. C., and Olcott, C. T.: Am. J. Path. **10**:81, 1934.
2. Ritchie, G., and Meyer, O. O.: Arch. Path. **22**:729, 1936.
3. Chevrel, F.: Ann. d'anat. path. **14**:297, 1927.
4. Epstein, E.: Ergebn. d. allg. Path. u. path. Anat. **33**:280, 1937.

was 1,100. A differential count could not be made, as the smear showed only an occasional mature polymorphonuclear neutrophil. These cells showed toxic changes. No immaturity was noted. The red cells showed poikilocytosis. There was no evidence of regeneration of red cells. The urine showed no abnormal features. The Kahn flocculation test was negative. The patient was given two blood transfusions together with iron and liver extract but showed no improvement. A red cell count on September 20 was 1,320,000, with 3.7 Gm. of hemoglobin per hundred cubic centimeters of blood. His white cell count was 400. He gradually grew weaker, bronchopneumonia developed, and he died on September 21. The clinical impression was that of aplastic anemia of undetermined nature. The possibility of aleukemic leukemia was considered because of the splenomegaly.

Autopsy.—Postmortem examination was done by one of us (A. J. Hertzog) one hour after death.

The body was 185 cm. in length and was well developed but emaciated; it was estimated to weigh 130 pounds (59 Kg.). There were petechiae in the skin over the abdomen and anterior part of the chest. The forearms showed venipuncture wounds. Rigor was absent. There was slight posterior hypostasis. There was grade 3 edema of both feet and ankles. There was no cyanosis or jaundice. The pupils were equal and measured 6 mm.

The peritoneal cavity contained 250 cc. of straw-colored fluid. The subcutaneous fat had a lemon yellow color. The peritoneal surfaces were smooth and glistening. The appendix was retrocecal and measured 7 cm. in length. The liver was large and extended 5 cm. below the costal edge. The spleen was greatly enlarged. The diaphragm arched to the fifth rib on the right and to the fifth interspace on the left.

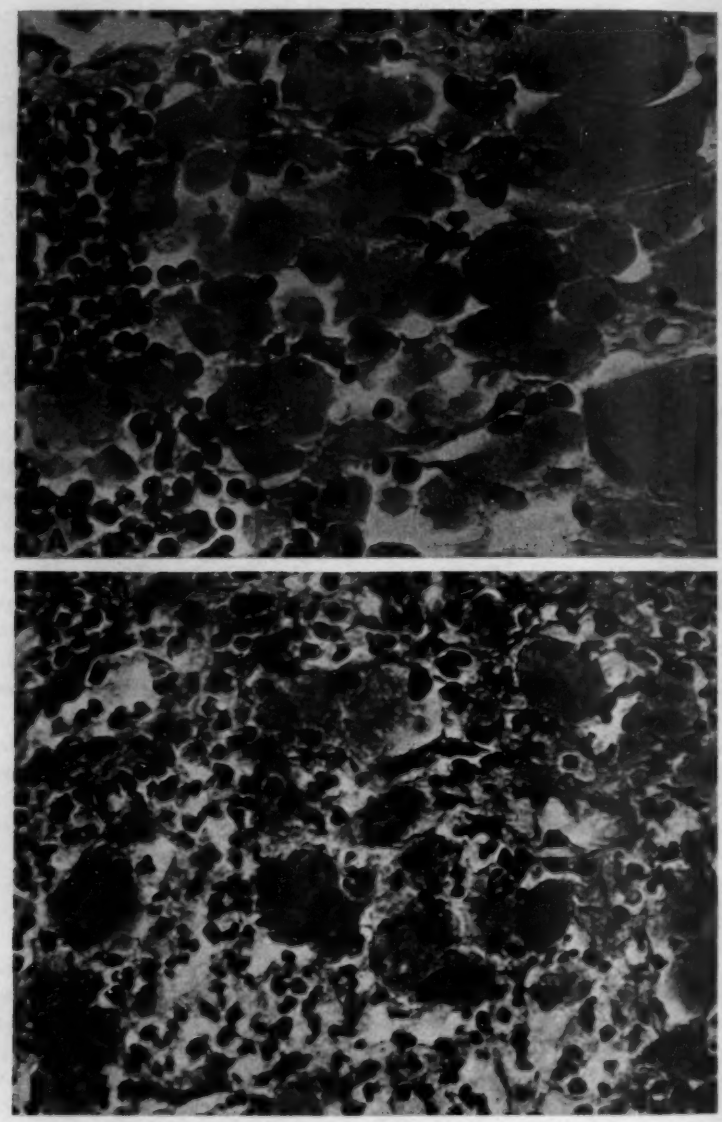
The right pleural cavity contained 1,000 cc. of cloudy brown fluid. There were fresh adhesions between the lateral surface of the lower lobe of the right lung and the wall of the chest. The left pleural cavity contained no fluid or adhesions. The pericardial sac contained a few cubic centimeters of straw-colored fluid. The walls appeared normal. The pulmonary artery showed no emboli.

The heart weighed 400 Gm. It was pale brown and had a peculiar rounded appearance. The musculature was very flabby. The epicardial surfaces appeared normal. The auricular appendages, endocardium and valves appeared normal. The septum was pale brown and showed no streaking. The root of the aorta appeared normal. The coronary arteries showed a minimum of sclerosis.

The right lung weighed 700 Gm. It was purplish gray. The consistence of the lower lobe was increased. The pleural surface was covered by fresh fibrinous adhesions. On section the lung was dark red. In the lower lobe of the right lung were nodular areas of bronchopneumonia. The remaining portions of the lung showed nothing of note except edema. There was no evidence of tuberculosis. The right hilar nodes were greatly enlarged, grayish black and rather soft. The left lung weighed 650 Gm. and was mottled gray. The consistence was somewhat increased, owing to partial atelectasis. On section it was pinkish gray and showed slight edema. There was no evidence of tuberculosis. The left hilar nodes were not enlarged.

The spleen weighed 1,100 Gm. It was purplish blue. There was a small cortical infarct, measuring 3 cm. in diameter. The capsule was tense, and on section the spleen was dark red. The pulp was markedly congested. The consistence was increased.

The liver weighed 3,400 Gm. It was pale brown. On section it was pale brown and showed no mottling. The markings were distinct. The gallbladder and bile ducts appeared normal.



A, photomicrograph of a lymph node showing large cells filled with lipoid. *B*, photomicrograph of spleen showing clusters of large clear reticular cells.

The esophagus appeared normal. The stomach contained a few cubic centimeters of brownish fluid. The walls appeared normal. The small bowel, colon and rectum showed nothing of note. The pancreas and adrenals appeared normal.

The right kidney weighed 150 Gm. and the left 175 Gm. The capsules stripped easily, revealing pale brown smooth surfaces. On section the cortex and medulla were of normal thickness. The pelves and ureters appeared normal. The bladder contained some straw-colored urine.

The prostate showed grade 1 hypertrophy. The testes appeared normal. The aorta showed grade 1 atherosclerosis.

The organs of the neck appeared normal. The mesenteric lymph nodes were greatly enlarged and on section had a pale, soft appearance.

The bone marrow of the shaft of the femur was red and hyperplastic. The bone marrow of the ribs was red.

The brain and spinal cord were not examined.

Microscopic Observations.—(*a*) Lymph Nodes: The normal architecture was entirely obliterated. There was hyperplasia of the reticulum in the form of large pale foamy cells (*A* in figure). When frozen sections were stained with sudan III, the large cells were found to be filled with droplets of fat of an orange red color. The fat droplets were stained blue with nile blue sulfate and were therefore interpreted as lipoid. Some portions of the nodes showed necrosis with many polymorphonuclear neutrophils.

(*b*) Spleen: The splenic pulp was markedly congested. The sinusoids were dilated. The follicles were small and indistinct. Throughout the pulp there was hyperplasia of the reticulum in the form of clusters of large clear foamy cells (*B* in figure). The fat droplets in these cells stained easily with sudan III and nile blue sulfate.

(*c*) Liver: The liver cord cells contained many large fat droplets as seen in extensive fatty metamorphosis. In addition, near the portal areas were a number of large foam cells similar to those seen in the spleen and lymph nodes.

(*d*) Lungs: The alveoli contained a large amount of serum and fibrin, together with large clumps of bacteria. There was complete absence of cellular exudate.

(*e*) Kidneys: The glomeruli, tubules and blood vessels showed no abnormalities.

(*f*) Thyroid and Adrenals: The sections showed nothing of note.

(*g*) Bone Marrow from Ribs and Femur: Sections showed hypoplastic marrow with a few clumps of large foam cells. Imprints stained with Wright and Giemsa stains showed, likewise, hypoplastic marrow with a deficiency of both red cells and granulocytes. A number of normoblasts, leukoblasts and myelocytes were seen. There were no megaloblasts. No foam cells could be seen on the imprints.

Chemical Analysis.—Portions of the lymph nodes and spleen were ground into dry powder. An alcohol-ether extract was obtained from each. This was tested for cholesterol according to the method of Bloor. The cholesterol content of the lymph nodes was 1.71 Gm. and of the spleen 1.95 Gm. per hundred grams. Epstein[5] reported the normal cholesterol content of the spleen to be 0.62 Gm. per hundred grams of dry powder.

COMMENT

The condition described clearly belongs to that group of reticuloendothelial disturbances which is associated with pathologic storage of lipoid. In clinical course and histologic features it resembles reticuloendotheliosis, but in the widespread storage of cholesterol in foam cells it bears a striking resemblance to Hand-Schüller-Christian disease. Inasmuch as the latter disease is now regarded as a disease of the

reticuloendothelial system, the disease described may be interpreted as a transition between Hand-Schüller-Christian disease and malignant lymphoblastoma of the reticuloendothelial type. It may also be interpreted as an atypical form of Hand-Schüller-Christian disease.

It is to be expected that occasionally one will encounter a condition that presents characteristics common to more than one of the recognized syndromes. Epstein [5] described a case in which, in addition to multiple xanthomas, free cholesterol was scattered throughout the brain. Chevrel [3] reported a case in which properties were presented which were common to both Gaucher and Niemann-Pick disease.

SUMMARY

The case is reported of a 54 year old man in whom fatal aplastic anemia developed in association with marked splenomegaly. At autopsy there was widespread storage of a lipoid within the cells of the reticuloendothelial system, especially of the lymph nodes and spleen. This lipoid proved to be cholesterol.

5. Epstein, E.: Virchows Arch. f. path. Anat. **298**:430, 1937.

RUPTURED DIVERTICULUM OF THE STOMACH IN A NEWBORN INFANT, ASSOCIATED WITH CONGENITAL MEMBRANE OCCLUDING THE DUODENUM

HENRY BRODY, M.D., NEW YORK

A unique combination of anomalies found in a 6 day old infant seems to be of sufficient interest to warrant reporting.

Martin [1] collected reports of 125 cases of pulsion diverticulum of the stomach, occurring from embryonic life to senescence. Since then a number of other reports have appeared.[2] No case of rupture of a diverticulum has been reported.

Kellogg and Collins [3] collected reports of 96 cases of congenital duodenal obstruction from the literature to 1933. A number of cases have since been reported.[4] Fourteen cases are similar to the one reported here, the obstruction being due to a membrane, complete or with fenestration, situated in the region of the entrance of the common bile duct.

REPORT OF A CASE

A normal-appearing newborn boy weighing 6 pounds 1½ ounces (2,764 Gm.) was placed on a formula supplementing breast feeding. He took feedings fairly well but regurgitated often during the first four days of life. He passed meconium, followed on the third day by dark brown stool. On the fourth day the infant became jaundiced. On the fifth day he vomited light brown fluid containing blood clot. His temperature was 101 F. The vomiting of blood-tinged fluid continued the following day. His blood showed 5,700,000 red blood cells and 5,600 white blood cells per cubic millimeter. The hemoglobin was 144 per cent. The differential count was normal, with no abnormal cells. The platelets numbered 347,000. The bleeding and the clotting time were within normal limits. In spite of the normal blood findings, and largely because of a history of "bleeders" in the family, a diagnosis of hemorrhagic disease of the newborn was made. A transfusion of 80 cc. was given. The infant continued to vomit blood-stained fluid, the temperature rapidly mounted to 105 F., and death occurred during the sixth day of life.

From the Department of Pathology, Beth Israel Hospital.

1. Martin, L.: Ann. Int. Med. **10**:447, 1936.

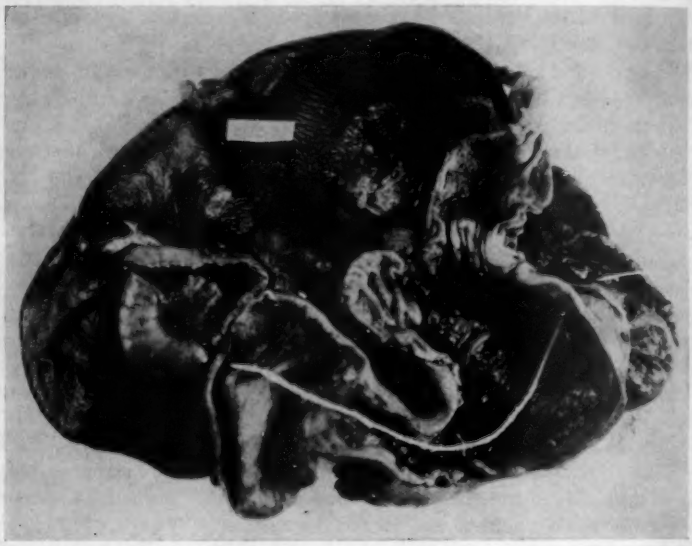
2. Hess, J. H., and Saphir, O.: J. Pediat. **6**:1, 1935. Rivers, A. B.; Stevens, G. A., and Kirklin, B. R.: Surg., Gynec. & Obst. **60**:106, 1935. Cheney, G., and Newell, R. R.: Am. J. Digest. Dis. & Nutrition **3**:920, 1936. Brown, P. W., and Priestley, J. T.: Proc. Staff Meet., Mayo Clin. **13**:270, 1938.

3. Kellogg, E. L., and Collins, J. T.: Am. J. Surg. **30**:369, 1935.

4. Ladd, W. E.: J. A. M. A. **101**:1453, 1934. Walker, T. D., Jr.; Falkener, W. W., and Horsley, J. S.: Virginia M. Monthly **62**:141, 1935. Bonar, T. G. D.: Lancet **2**:822, 1935. Regnier, E. A.: Minnesota Med. **18**:60, 1935. Greenblatt, A.: Brit. M. J. **2**:840, 1935. Cesaris Demel, V.: Pathologica **27**:533, 1935. Niosi, G. S.: ibid. **28**:414, 1936.

Autopsy.—Only the pertinent anatomic findings will be described. The abdomen was markedly distended. When the peritoneum was opened, a considerable amount of air under pressure escaped. The abdominal cavity contained about 200 cc. of partly bloody, partly brownish-black fluid and semifluid, somewhat foul-smelling material, containing numbers of large clots. The omentum was rolled and lightly applied transversely to the anterior abdominal wall, thus confining most of the material to the left upper quadrant. The intestinal loops were cyanotic, and the serosal surfaces of the diaphragm, liver and mesenteries were deeply hyperemic and in part covered with a thin, dirty brown exudate.

When the mass of material was removed, there was seen, projecting from the left border of the stomach, a thin-walled sac, which after fixation measured 4 by 3 by 3 cm. The upper border of the diverticulum was 1 cm. below the esophageal-cardiac junction, and the diverticulum extended for 4 cm. along the



Liver raised to show opened esophagus, stomach and duodenum. A white thread passes through the perforation in the diverticulum and lies in the lumen of the stomach and duodenum, reaching the point of atresia. All the structures proximal to the atresia are dilated.

greater curvature, reaching a point 5 cm. above the pyloric sphincter. Along the anterior surface of the diverticulum was an irregular rent, of which the antemortem dimensions could not be determined because of its extreme friability. After fixation (and with some postmortem tearing) the aperture was 2.5 cm. in the greatest diameter. Grossly no muscle could be recognized in the thin membrane forming the wall of the diverticulum.

The lower portion of the esophagus was dilated. After fixation its diameter at the widest point, 1.5 cm. above the cardia, was 2.5 cm. The stomach was moderately distended, the pyloric sphincter relaxed and wide. The first portion of the duodenum was markedly distended. In the fixed state its greatest circumference was 4.5 cm. Two and five-tenths centimeters below the pyloric sphincter

the duodenal lumen became suddenly narrowed, and at the level of the ampulla of Vater it was completely occluded. The common bile duct was slightly distended, measuring after fixation 2.5 mm. in diameter. A probe readily passed from above to the point of atresia in the duodenal wall but not into the duodenal lumen of either the upper or the lower segment. However, bile was present below the point of atresia, while a chemical test failed to reveal any in the contents above. The duodenum below the point of atresia was of normal diameter. The remainder of the small intestine was slightly narrow and contained light green semisolid material. The large intestine was also of rather narrow diameter and contained semisoft material stained light brown and light green. The intestinal tract showed no other abnormalities.

The bile ducts, except as described, and the gallbladder were normal. The liver appeared normal, without evidence of bile stasis, Multiple cross sections of the pancreas revealed the pancreatic duct not distended but its wall stained a deep green. The gland appeared otherwise normal.

Microscopic Examination.—Sections were taken through the stomach to include both normal stomach and a portion of the diverticulum. The muscular layer ended abruptly, and in the region of the diverticulum there was present only mucosa, submucosa and serosa. The serosa was covered with an exudate composed of red blood cells, fibrin, necrotic leukocytes and bacteria. The underlying submucosa was edematous and congested but showed no inflammatory exudate. In one section the free end of the muscle had pulled away from the submucosa to form a small protuberance beneath the serosa. It had carried with it part of the submucosa, and in this region there was some hemorrhage. In the same section the mucosa in the region of the diverticulum appeared stretched, and further evidence of this was the thinning of the muscularis mucosae as the edge of the section was approached.

The region of the atresia was studied in serial sections. Longitudinal section showed that the atresia was due to a membrane made up of double layers of normal wall, lying back to back, with some aberrant pancreatic tissue occurring at the base and extending for a short distance between the layers of the membrane. The pancreatic duct and the common bile duct could be traced through this area into the lower segment.

The liver showed no evidence of bile stasis.

Several pancreatic ducts contained brown pigment and bacteria. No inflammatory reaction was present. The ducts were not distended. There was no change in the acinar or islet tissue.

COMMENT

The problem arises whether there is any necessary and causal relation between the congenital obstruction of the duodenum and the diverticulum of the stomach. As Martin [1] pointed out in his paper, the most common site for a gastric diverticulum is near the cardiac opening, and it is in this region that the musculature of the stomach is most poorly developed. In the present case the diverticulum is along the greater curvature, somewhat below the cardia. It is most probable that this diverticulum is congenital and that it is independent of the atresia of the duodenum. It is difficult to believe that the obstruction within the duodenum served to raise the intragastric pressure during antenatal life sufficiently to have produced thinning of the stomach wall and to have done so not at a natural point of weakness. Busch,[5] in a study

5. Busch, M.: Frankfurt. Ztschr. f. Path. **30**:30, 1924.

of rupture of the stomach, pointed out that the usual site of rupture is along the lesser curvature, close to the cardia. Further, such a diverticulum has never been reported in association with congenital obstruction of the intestine.

However, the dilatation of the duodenum proximal to the point of atresia and the dilatation of the stomach and of the esophagus are evidence of an increase in pressure in this portion of the gastrointestinal tract. It seems reasonable to assume that the increase started with birth and the taking of food. How much of a role antenatal swallowing of amniotic fluid and antenatal secretion of digestive juices may have played is impossible to evaluate. It seems probable that the increase in pressure was the immediate cause of the rupture of the congenital diverticulum and that this was signaled clinically by the appearance of blood-streaked vomitus.

The only mention in the literature of a case in which congenital stenosis of the duodenum caused rupture proximally is that of Cannon and Halpert.[6] They described, in an 8½ year old girl, partial duodenal stenosis caused by an incomplete membrane at the level of the entrance of the common bile duct, with hypertrophy of the wall of the stomach secondary to the stenosis. In the membrane was a slitlike orifice. Acute obstruction of the lumen was produced by ingested vegetable leaves. Rupture of the stomach followed in several hours. The rupture occurred 1 to 2 cm. from the cardia and was brought about by unusually vigorous treatment with irritant enemas of excessive amount.

SUMMARY

A congenital diverticulum of the stomach associated with atresia of the midportion of the duodenum is reported. Rupture of the diverticulum occurred on the sixth day of life. No previous case of a ruptured gastric diverticulum has been found recorded in the literature.

6. Cannon, P. R., and Halpert, B.: Arch. Path. **8**:611, 1929.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—In the school of medicine of the University of Colorado, W. C. Black has been promoted to professor and K. T. Neuberger to assistant professor of pathology; R. M. Mulligan has been appointed instructor in pathology and A. S. Lazarus instructor in bacteriology.

C. F. Graham has been appointed associate professor in the department of bacteriology and pathology in Albany Medical College.

Edgar H. Norris, professor of pathology in the college of medicine of Wayne University, Detroit, has been elected dean of the college.

As provided by recent legislation, Perry J. Melnick has been appointed pathologist to the cancer diagnosis division of the department of public health of the State of Illinois.

C. C. Bass, dean and professor of experimental medicine in the school of medicine of Tulane University, New Orleans, has retired, having reached the age limit.

Awards.—The Eli Lilly Award of a medal and $1,000 was presented to John G. Kidd, of the Rockefeller Institute for Medical Research, by the Society of American Bacteriologists at its last annual meeting. This award is given for outstanding work in bacteriology or immunology by a person under 31 years of age.

At the meeting of the American Society of Tropical Medicine on Nov. 21, 1939, the Walter Reed medal was awarded to William B. Castle, professor of medicine, Harvard Medical School, Boston, for work on sprue and related anemias.

Society News.—The annual meeting of the Society of American Bacteriologists was held in New Haven, Conn., Dec. 28-30, 1938. This was the fortieth anniversary of the society, which was founded in New Haven. Charles Thom, in charge of soil microbiology in the United States Department of Agriculture, was elected president.

International Salmonella Center.—This center, established at the State Serum Institute in Copenhagen, supplies Salmonella centers elsewhere with the cultures and serums necessary for diagnosis. Thirty-seven such centers have been established. These centers will study doubtful cultures of Salmonella without any charges. The centers in this country are: Beth Israel Hospital (Dr. F. Schiff), New York; New York State Department of Health (Dr. A. B. Wadsworth), Albany, N. Y.; University of Kentucky (Dr. A. R. Edwards, department of animal pathology), Lexington, Ky.

Microfilm Sets of Periodicals.—The Committee on Scientific Aids to Learning (President Conant of Harvard, chairman) has made a grant to cover the cost of making a microfilm master negative, on the most expensive film, of sets of volumes of scientific and learned journals. This permits the nonprofit Bibliofilm Service to supply microfilm copies at the sole positive copy cost, namely, 1 cent per page for odd volumes and ½ cent per page for any properly copyable ten or more consecutive volumes. The number of pages will be estimated on request to: American Documentation Institute, care of offices of Science Service, 2101 Constitution Avenue, Washington, D. C.

Legislation for Blood Grouping Tests.—Laws providing for blood grouping tests in cases of disputed parentage are now in force in New York, Wisconsin, Ohio and New Jersey. It has been recommended that laws of this kind should include provision for the payment of experts for their services in such cases by the jurisdiction concerned, because in so many instances the men involved are unable to pay any fees.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

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Experimental Pathology and Pathologic Physiology

The Earliest Known Reference to the Heart and Circulation. W. W. Hamburger, Am. Heart J. **17**:259, 1939.

This is a review of "the earliest known reference to the heart and circulation" as recorded in the Edwin Smith Surgical Papyrus, of about 3,000 B. C. The paper is of interest because it illustrates "how very distant and far reaching are the roots of our knowledge in the biological sciences."

Effects of Diphtheria Toxin on the Heart. R. W. Boyle, C. H. McDonald and A. F. DeGroat, Am. Heart J. **18**:201, 1939.

The hearts of 7 dogs which had been given varying doses of diphtheria toxin were studied with respect to electrocardiographic behavior, distribution of glycogen and pathologic changes. The electrocardiographic changes noted consisted of various degrees of block and abnormalities of the T wave. Parenchymatous degeneration of a mild degree was invariably present. The most characteristic change was the presence of hyaline streaks scattered through the muscle fibers. Glycogen—taking the heart as a whole—showed a 10 per cent rise in the hearts of dogs given a slowly lethal injection of diphtheria toxin. A rise was noted in the two atria and the two ventricles, and no change in the septum. A quickly lethal dose failed to produce any effects on cardiac glycogen. In view of the increases in cardiac glycogen which occur during starvation and of the self-imposed starvation (anorexia) always present in severe diphtheritic toxemia, it is suggested that the moderate rise encountered in such cases is the result of starvation rather than of any specific effect of the diphtheria toxin on cardiac glycogen.

From Authors' Summary.

Mineral Appetite of Parathyroidectomized Rats. C. P. Richter and J. F. Eckert, Am. J. M. Sc. **198**:9, 1939.

Parathyroidectomized rats showed a markedly increased appetite for solutions of calcium salts (lactate, acetate, gluconate and nitrate) and an aversion toward a solution of disodium phosphate (dibasic sodium phosphate). Parathyroidectomized rats also showed an increased appetite for solutions of strontium and magnesium salts. These results agree with present knowledge concerning the disturbance of calcium and phosphorus metabolism following parathyroidectomy. The decreased mortality and the alleviation of symptoms of deficiency in parathyroidectomized rats given access to solutions of calcium salts add further evidence that rats have an ability to make selections conducive to their well-being.

From Authors' Summary.

Effect of Diet on Nephritis in Rats. J. E. Smadel and L. E. Farr, Am. J. Path. **15**:199, 1939.

In rats of the so-called Whelan strain the chronic nephritis which follows the administration of antikidney serum can be markedly influenced by isocaloric diets containing different proportions of protein and carbohydrate.

From Authors' Conclusions.

EXPERIMENTAL PRODUCTION OF NEUTROPENIA WITH AMINOPYRINE. E. M. BUTT, A. H. HOFFMAN and S. N. SOLL, Arch. Int. Med. **64**:26, 1939.

It has been shown in these experiments on dogs that aminopyrine has a toxic effect on the bone marrow when administered orally in large doses. The end result is one of severe aplasia of the marrow rather than of hyperplasia with "maturation arrest." Neutropenia was noted in 1 of the 2 dogs having markedly aplastic bone marrow. A summary of the literature on the experimental production of malignant neutropenia is presented.

FROM AUTHORS' SUMMARY.

CAUSES OF THE CESSATION OF GROWTH OF FIBROBLASTS IN EMBRYO JUICE. L. E. BAKER, J. Exper. Med. **69**:625, 1939.

Experiments designed to ascertain the reason for the cessation of growth of cardiac fibroblasts when they are cultivated in a plasma coagulum with embryo juice as nutrient fluid have shown that it is due, first, to the gradual removal of serum from the coagulum and, second, to an insufficiency of embryo juice. In a medium containing embryo extract at 66 per cent concentration and serum at 8 per cent concentration growth continued until the entire coagulum in a 3.5 cm. flask was covered with tissue. The serum is needed to furnish additional nutriment and also to prevent digestion of the coagulum.

FROM AUTHOR'S SUMMARY.

ENHANCING EFFECT OF AZOPROTEINS ON LESIONS. A. CLAUDE, J. Exper. Med. **69**:641, 1939.

It is known that solutions of azoproteins, like testicular extracts, possess the property of causing particles to spread through the dermis. The present work shows that azoproteins exhibit, like testicular extracts, the power to increase the size of the lesions caused by virus in the skin of rabbits and the size of tumors in chickens. The results indicate that the extent of the lesion is roughly proportional to the spreading power of the solution. This suggests that the spread of the infective material over a larger area of skin is directly responsible for the enhancing effect. The production of extensive lesions by means of spreading agents may have a practical value when large amounts of working material are needed.

FROM AUTHOR'S SUMMARY.

EXPERIMENTAL STUDY OF ATROPHY OF TISSUES AND ORGANS. G. CAVALLI, Ann. d'anat. path. **13**:691, 1936.

In three groups of rabbits (1) the renal artery, (2) the renal vein and (3) both the artery and the vein were ligated on one side, and the subsequent changes were studied histologically at weekly intervals. At first there was marked swelling of the kidney, especially when the renal vein alone was tied, due to edema and interstitial hemorrhage. Shrinkage then began, and the kidney became progressively smaller and firmer. At the second week marked degeneration of the tubular epithelium was seen, followed by resorption of necrotic material and organization of the kidney by appearance of granulation tissue and proliferation of connective tissue. Many small newly formed blood vessels were seen, apparently derived from collateral sources, i. e., capsular, adrenal, ureteral and lumbar branches. There were no regenerative processes. In the third week fibrosis was advanced, and metaplastic bone formation began. Possible reasons for the occurrence of metaplastic ossification are advanced. The article also contains a historical survey of the subject of ligation of renal vessels.

PERRY J. MELNICK.

COMPARATIVE STUDY OF PULMONARY LESIONS OF NERVOUS ORIGIN. J. TINEL, G. UNGAR and J. BRINCOURT, Ann. d'anat. path. **13**:898, 1936.

The lungs of dogs and rabbits were examined after section and after section plus electrical stimulation of the vagus nerves, phrenic nerves and carotid sinus.

Section of the vagus nerves, even without electrical stimulation, resulted in a picture resembling bilateral bronchopneumonia, with hepatization and with the presence of an abundant cellular exudate in the alveoli. Electrical stimulation of the phrenic nerves or of the posterior cervical nerve roots or of the carotid sinus resulted in marked edema and hyperemia of the alveolar walls and extravasation of red cells, a picture resembling hemorrhagic infarcts. It is suggested that the nervous system may play a role in the production of pulmonary edema and congestion, pneumonia and pulmonary infarcts.

PERRY J. MELNICK.

NEPHROGENIC OSTEODYSTROPHY. E. RUTISHAUSER, Ann. d'anat. path. **13**:999, 1936.

By inserting many glass needles or copper wires into the kidneys of rabbits Rutishauser was able to produce chronic nephritis of a type which causes a disturbance of calcium and phosphorus metabolism and results in osteodystrophy resembling von Recklinghausen's osteitis fibrosa. Ten rabbits were used. One of these is described in detail as an example. Before operation the blood calcium was 11.3 and the blood phosphorus 6.4 mg. per hundred cubic centimeters. Ninety days later the figures were 16.7 and 4.5, and one hundred and thirty-eight days later, 16.9 and 6.2, respectively. One hundred and forty-nine days after operation the rabbit died in a state of emaciation. At autopsy there was found chronic cicatrizing nephritis about the glass needles. Calcium deposits (calcium "metastases") were found in the renal tubules, stomach and lungs (i. e., in areas where there was local alkalosis consequent to the excretion of acid substances). The bones were found partially decalcified, and the microscopic picture was that of osteitis fibrosa. The parathyroid glands were somewhat hypertrophied.

PERRY J. MELNICK.

ORIGIN OF OBLITERATING ARTERITIS AND ITS PRODUCTION FOLLOWING REPEATED GRAFTING OF ADRENAL GLANDS. R. LERICHE and F. FROEHLICH, Ann. d'anat. path. **13**:1039, 1936.

Adrenal glands were repeatedly grafted into 5 rabbits; from 6 to 40 grafts were performed over periods varying from twenty-seven days to six months and seven days. In all of these animals changes were found in the arteries on microscopic examination. These changes consisted chiefly of marked thickening and hypertrophy of the intima and media, leading to stenosis and sometimes to complete obliteration of the lumen. The findings in each of the 5 animals are described in detail. The authors conclude that the adrenal gland exerts an influence in the production of obliterating arteritis.

PERRY J. MELNICK.

Pathologic Anatomy

CEREBRAL VASCULAR DISEASE ACCOMPANYING SICKLE CELL ANEMIA. W. H. BRIDGERS, Am. J. Path. **15**:353, 1939.

A study of 2 patients with sickle cell anemia (the first was an adult and the second a child) has shown that the disorder may first become manifest through the appearance of signs and symptoms indicative of cerebral vascular disease. The clinical features in such patients lead to the diagnosis of either cerebral vascular thrombosis or intracranial hemorrhages. The pathologic changes seen in the first of the patients establish the fact that in sickle cell anemia the large subarachnoid cerebral arteries may undergo gradual obliteration with final complete closure through the operation of a process identical with that which results in occlusion of the splenic arteries. This process is one of endarterial intimal proliferation and not of thrombosis. In the second patient autopsy showed that another vascular process, quite different from endarterial intimal proliferation, also occurs in sickle cell anemia. This process develops in connection with the

small intracerebral vessels and may result in multiple focal necroses and hemorrhages in the brain, in contrast with the large infarcts that characterize the proliferative obstructive process in the larger arteries. The nature of this second process is not clear.

FROM AUTHOR'S SUMMARY.

PATHOLOGIC CHANGES FOLLOWING THERAPEUTIC HYPERTHERMIA. L. LICHTENSTEIN, Am. J. Path. **15**:363, 1939.

A description is given of the changes observed at autopsy in a case of uncontrollable hyperpyrexia (109 F.) ensuing on hyperthermia treatment for arthritis of the finger joints. The hyperpyrexia (which developed in the course of the third of a series of treatments) was associated with coma and respiratory failure, and the patient died about twenty-five hours after the fever was initiated. In this case the significant pathologic changes were: (1) multiple punctate hemorrhages and necrobiosis in the gray matter of the cerebral cortex; (2) hemorrhage in the left internal capsule; (3) thrombosis of venules and capillaries in the cerebral cortex and internal capsule; (4) cerebral congestion and edema; (5) infarction of the kidneys and spleen; (6) marked hepatic degeneration and edema, and (7) pulmonary congestion, hemorrhage and edema. The changes found in this case have been correlated with, and discussed in relation to, those in 9 cases previously recorded in the literature. Certain of the changes seen in the case reported here, notably the vascular lesions, have not hitherto been described in connection with fatalities following fever therapy. Specifically there seems to be no previous description of the thrombosis of venules and capillaries in affected portions of the brain and of the infarcts in the kidneys and spleen apparently due to focal necroses of small arterial branches in these organs. The principal complications and sequelae of hyperthermia—especially its effects on the brain, blood vessels and liver—are indicated. Attention is also drawn to the fact that the reactions to therapeutic hyperthermia are sometimes serious even when they are not fatal.

FROM AUTHOR'S SUMMARY.

MYOCARDIAL INFARCTION WITHOUT SIGNIFICANT LESIONS OF THE CORONARY ARTERIES. H. GROSS and W. H. STERNBERG, Arch. Int. Med. **64**:249, 1939.

Fifteen cases of myocardial infarction without demonstrable occlusion of the coronary arteries were studied. Arteriosclerosis of the coronary arteries was minimal. In 1 case all the major vessels were sectioned serially. A variety of physiologic mechanisms which might account for myocardial damage in the absence of vascular occlusion is outlined. These may be (1) mechanical, (2) reflex or (3) humoral: Mechanical factors include temporary falls in intraaortic blood pressure, tachycardia, phasic variations of the coronary flow and cardiac hypertrophy. Changes either in the number of formed elements or in the viscosity of the blood, such as are seen in anemia and polycythemia, are contributory factors. Reflex factors may produce anoxia of the myocardium from failure of adequate compensatory dilatation of the coronary arteries or from coronary constriction. The stimuli for these reflexes may arise either in the heart and its associated structures or in other parts of the body. Humoral factors and possibly other agents, including acetylcholine, epinephrine and pitressin, also alter the caliber of the coronary arteries. The presence of hypertension in 13 of the 15 cases is of great interest. Vasoconstrictor phenomena are common in hypertension, and it is conceivable that extreme coronary vasoconstriction may have played a role. The underlying cause of the anginal seizure, whether transitory or associated with myocardial infarction, is ischemia of the myocardium. Similarly, the electrocardiographic changes in both instances are also the result of myocardial ischemia. The duration of ischemia will determine whether the cardiographic and myocardial changes are reversible or not. Though the type of case reported is infrequent, it is nevertheless of importance, since similar physiologic factors must also play a role in many cases of ordinary coronary disease and myocardial damage.

FROM AUTHORS' SUMMARY.

HISTOLOGIC CHANGES IN THE NERVOUS SYSTEM IN CASES OF PEPTIC ULCER. A. R. VONDERAHE, Arch. Neurol. & Psychiat. **41**:872, 1939.

The effect of peptic ulcer (duodenal, gastric or both) on the brain or vice versa was investigated by Vonderahe on extensive clinicopathologic material. In the 14 cases of peptic ulcer studied, hemorrhages, frequently punctate, were found in certain areas of the diencephalon (thalamus, hypothalamus) and in the dorsal motor nucleus of the vagus nerve, usually on one side. Control studies of the diencephalon (in cases of malignant hypertension, puerperal septicemia, paresis, carcinoma of the lungs and diabetes mellitus) disclosed no evidence of hemorrhages into the aforementioned regions, though such hemorrhages were found in association with other conditions, especially irritative disease of the abdominal viscera and peritoneum and, in some cases, diabetes mellitus. On the whole, hemorrhages in specific areas of the thalamus and hypothalamus are, according to Vonderahe, a consistent observation in cases of peptic ulcer. The medulla was investigated in a variety of pathologic conditions (disease of the heart, kidneys, lungs and other organs), and neither hemorrhages nor diapedesis was found to occur in the motor nucleus of the vagus nerve. Only in the presence of peptic ulcer were punctate hemorrhages disclosed with regularity in this nucleus. In 20 per cent of cases of tumor of the brain there was evidence of incipient or well advanced gastrointestinal ulceration, and in 11 of 56 cases of gastrointestinal ulceration gross nonneoplastic lesions of the brain and the meninges were present. The hemorrhages are supposed to be due to vasomotor alterations in the cerebral blood vessels as the result of implication and excessive stimulation of the visceral afferent components of the vagus and sympathetic nerves.

GEORGE B. HASSIN.

HISTOLOGIC STUDY OF OSLER'S NODES. L. CORNIL, M. MOSINGER and A. X. JOUVE, Ann. d'anat. path. **13**:675, 1936.

The authors made a histologic study of a subcutaneous rheumatic node removed twenty-four hours after its appearance. The essential finding was an injury of the endothelium of the smaller blood vessels. Swelling, vacuolation and sometimes necrosis of the endothelium were seen, with occasional secondary thrombosis. Usually, however, proliferation of the endothelial cells occurred, sometimes sufficient to occlude the lumen. Necrosis about the blood vessels and marked cellular infiltration of the adventitia were also seen. Bacteria were not found. The authors do not believe that these lesions are embolic but consider them as of toxic or hyperergic origin, and they compare them to Fraenkel's nodules in typhus fever and to the rose spots of typhoid. (The descriptions and photomicrographs give the impression, in the light of recent concepts regarding the nature of rheumatism and in view of the work of Rössle, Klinge and many others, that these lesions should probably be considered as allergic granulomas.)

PERRY J. MELNICK.

CYTOLOGY OF THE VASCULAR AND MESENCHYMAL LESIONS IN TYPHUS FEVER AND THEIR SIGNIFICANCE. E. CRACIUN, Ann. d'anat. path. **13**:817, 1936.

In typhus fever the cutaneous nodules, which occur about the arterioles in the reticular layer of the dermis, first described by Fraenkel, have a specific histologic development. This consists in an endothelial injury (sometimes necrosis and sometimes proliferation and desquamation into the lumen), followed by a histiocytic infiltration of the wall of the blood vessel and of its adventitia. The specific character of Fraenkel's nodules has been confirmed by many. In this article Craciun, on the basis of a study of the observations made at 22 autopsies, concludes that this same specific character is found in the visceral lesions of typhus fever also. He gives a detailed description and discussion of the visceral lesions.

PERRY J. MELNICK.

ANATOMIC STUDY OF INTERSTITIAL ANEURYSMS OF THE LUNG. M. DELHOMME and M. IGLESIAS Y BETANCOURT, Ann. d'anat. path. **13**:961, 1936.

Of 4 cases of pulmonary tuberculosis in which death followed hemoptysis and in which an autopsy was made, 3 were studied in detail. The lungs were found to contain large hematomas enclosed in cavities. These cavities, however, had all the characteristics of aneurysms, having lamellar layers of fibrin at the periphery and afferent and efferent vessels. The authors present a theory of formation of these interstitial aneurysms on the basis of caseous involvement of a blood vessel with weakening of part of the wall and formation of an aneurysm.

PERRY J. MELNICK.

CENTERS OF ORIGIN AND MODES OF DEVELOPMENT OF VENOUS THROMBOSIS OF THE LOWER EXTREMITY. R. NEUMANN, Virchows Arch. f. path Anat. **301**: 708, 1938.

In 165 consecutive necropsies the venous system was thoroughly examined for thrombosis. Special attention was paid to the veins of the lower extremities, and the observations form the basis of this report. Eighty-four of the subjects were women. Eighty-one were men. The youngest was 17 years old; the oldest 88. Thrombosis was detected in 100 of the 165 subjects. The localization was as follows: plantar region, 71 per cent; internal malleolar region, 17 per cent; leg, 87 per cent; thigh, 22 per cent. Thrombosis limited to the veins of the thigh alone was not observed. Great importance is attached to the fact that the malleolar vesels, which connect the veins of the foot and the leg, were so infrequently involved even when both the plantar and the leg regions were seats of thrombosis. This leads to the conclusion that there are two independent centers of origin of thrombosis in the veins of the lower extremity: one in the plantar region, the other in the leg region. Two clinical types are recognized, depending on the center of origin: (1) a benign type, which has its center of origin in the veins of the leg and which is characterized by slow progression, an incidence that increases progressively with age and a tendency toward the occurrence of multiple nonfatal emboli in the lung; (2) a malignant type, which has its center of origin in the plantar region and is characterized by rapid progression of the thrombosis, occurrence at earlier ages, with no increase with age, and a tendency toward fulminating fatal embolism of the lung. In both types the source of emboli is a thrombus that has progressed to the veins of the thigh. In progressive ascending thrombosis segmentation results where ligaments, tendons and the like cross over veins. In thrombosis three factors are recognized: (1) a preparatory factor, usually endovascular; (2) a precipitating factor, which may be vascular, endovascular or perivascular, and (3) a localization factor, which is usually perivascular.

O. T. SCHULTZ.

ADAPTIVE CHANGES IN THE CRANIUM IN RELATION TO BRAIN GROWTH. J. ERDHEIM, Virchows Arch. f. path. Anat. **301**:763, 1938.

This article, written in Erdheim's lucid style, had evidently been completed before his death. He describes in detail the changes that occur in the skull in its growth and in its adaptation to the brain from fetal life to old age. It is constantly in a state of flux, which makes of the cranium a plastic structure in spite of its osseous nature. Three processes are interrelated and are in constant interplay with each other. These Erdheim terms *Anbau, Abbau* and *Umbau,* words that do not lend themselves to translation into single terms. They might be translated as "construction, or building," "destruction, or absorption," and "reconstruction, or rebuilding," although these words do not quite express the shades of meaning of the original. In these processes the dura, the epicranial periosteum and the endosteum of the diploe play a part. As the internal surface of the bone is resorbed by the dura, new bone is laid down by the diploe and epicranium. Increase in the size of the cranial cavity is not a simple matter of

expansion and growth of bone at the suture lines but a progressive rebuilding, so that the inner and outer tables and the diploe occupy a position relatively distant from that which they had in earlier life. Increase in the area of the flat bones occurs by a similar process of resorption and new formation of bone at the suture margins. Resorptive and reconstructive changes induced by the dura lead to modeling of the internal surface of the cranium. The rate at which the various processes proceed varies, and at times growth ceases, resulting in the formation of lines of cessation of growth, to which Erdheim had attached importance in previous communications. That the inner surface of the cranium is not modeled or molded to correspond to the sulci and convolutions of the surface of the brain is due to the fact that the pia-arachnoid with its subjacent fluid forms a smooth envelop which protects the dura against the apparent inequalities of the cerebral surface. Having established what he considers the normal course of events, Erdheim proceeds to discuss in three final sections three pathologic states in the light of his concept. In senile hyperostosis the volume of the cranial cavity must decrease to accommodate itself to the shrinking senile brain. An increase in the quantity of subdural liquid plays a part, but appositional bone formation by the dura is more important. Next the changes in the skull associated with increased intracranial pressure are described, especial attention being paid to the reconstructive alterations that occur when the pressure is permanently restored to normal by removal of the cause of the increase in pressure. The final section is devoted to *Lückenschädel,* in which the cranial juga are greatly increased and thickened and the digitate impressions are correspondingly widened and the bone over them thinned, to such a degree that perforation of the skull may occur. It appears that Erdheim had contemplated a study along similar lines of other pathologic changes in the cranial bones. O. T. Schultz.

Microbiology and Parasitology

Virus Pneumonia of Infants Secondary to Epidemic Infections. E. W. Goodpasture and others, Am. J. Dis. Child. **57**:997, 1939.

Five cases of a hitherto undescribed virus infection of the lungs of infants, which develops especially following measles and whooping cough, are recorded. The virus invasion usually appeared to be secondary and tended to pave the way for bacterial infection of the lungs. The presence of the virus was indicated by the occurrence of nuclear inclusions in epithelial cells of the trachea and bronchi and their mucous glands and in the alveolar epithelium. The affected cells rapidly became necrotic, with resultant ulceration of surfaces. The virus appeared to be different from that of herpes simplex and from the agent of the so-called inclusion disease of infants. Experimental inoculation of the infected lung tissue into rabbits, mice, opossums, chicken embryos and a Macacus rhesus monkey failed to establish the infection. From Authors' Summary.

Reaction of Killed Tubercle Bacilli. C. T. Olcott, Am. J. Path. **15**:287, 1939.

When heat-killed tubercle bacilli are injected into the peritoneal cavities of rabbits, tubercles and tuberculous tissue form more quickly and become more advanced in animals that have received preliminary intracutaneous or subcutaneous injections of killed tubercle bacilli. The difference is greatest three or four weeks after the intraperitoneal injection of the bacilli, when the lesions in prepared and in control animals are at their height. After five weeks no difference in the two groups is evident, and later the lesions in both regress. The extent of tuberculous lesions is greater in the prepared animals, which are both immunized and sensitized, than in their controls, but there is no close correlation between sensitization as measured by the tuberculin reaction and the extent of the tuberculous lesions. Heat-killed tubercle bacilli cause necrosis with the characteristics of caseation

both in control (unprepared) animals and in those that have received preparatory injections, but this type of lesion is more frequently found in the latter. Caseation is in general more advanced in sensitized animals, but there is no exact correlation between sensitization and caseation. When killed tubercle bacilli have been injected into the peritoneal cavity, tubercle-like nodules composed of epithelioid cells are often found in the retrosternal lymph nodes, spleen, liver and lungs of both immunized and control animals.

FROM AUTHOR'S SUMMARY.

SUBAXILLARY GLAND VIRUS OF GUINEA PIG. C. T. ROSENBUSCH and A. M. LUCAS, Am. J. Path. **15**:341, 1939.

There is some evidence that young guinea pigs are more resistant to the action of the submaxillary gland virus than full-grown animals of the same stock.

FROM AUTHORS' SUMMARY.

INDUSTRIAL DUSTS AND THE MORTALITY FROM PULMONARY DISEASE. A. J. LANZA and R. J. VANE, Am. Rev. Tuberc. **39**:419, 1939.

Early studies of occupational mortality focused attention on dust as a cause of respiratory diseases. Virtually all kinds of dusts were implicated. Present day studies point to the serious damage of lung tissue caused by a few dusts, notably silica and asbestos, and to the relatively little damage caused by many other dusts. Yet only qualified general conclusions are permitted except for silica. The death rate from tuberculosis among occupational groups who are freely exposed to silica so far exceeds those found for other groups as to leave little room for doubt that silica is implicated. With regard to American mortality from silicate and other inorganic dusts not containing free silica, data are meager. The effects of aluminum oxide, silicon carbide and other substances used in manufactured wheels are slight. British data show lower than average mortality from tuberculosis up to the age of 35, but substantially higher mortality from this disease after the age of 45, for a group of men exposed to inorganic dusts other than silica dust. The relationship between the inhalation of dust and acute pulmonary disease remains a field for further investigation.

H. J. CORPER.

BLASTOMYCOSIS. D. B. MARTIN and D. T. SMITH, Am. Rev. Tuberc. **39**:488, 1939.

American blastomycosis is a distinct clinical entity, caused specifically by Blastomyces dermatitidis. Two types of infection caused by this fungus are recognized clinically: (*a*) cutaneous blastomycosis, a chronic or subacute ulcerating process, usually responding to treatment with iodides or radiation, and (*b*) systemic blastomycosis, a highly fatal disease, characterized by pulmonary infection and widespread distribution of lesions. The disease is more common in males. The serum of heavily infected patients discloses the presence of antibodies. In some patients a condition of hypersensitiveness to the fungus develops, which diminishes in the terminal stages of the disease. The degree of hypersensitiveness can be estimated by cutaneous tests and is materially reduced by repeated injections of minute doses of heat-killed vaccine. In some cases potassium iodide is curative, but it is a dangerous drug to administer to patients allergic to the fungus. In systemic blastomycosis iodide therapy should be started only after the state of hypersensitiveness has been excluded by cutaneous tests or artificially reduced by therapeutic injections of vaccine.

H. J. CORPER.

EXPERIMENTAL AND CLINICAL GRANULOMA INGUINALE. R. B. GREENBLATT, R. B. DIENST, E. R. PUND and R. TORPIN, J. A. M. A. **113**:1109, 1939.

Granuloma inguinale was experimentally reproduced in 3 human beings but failed to develop in 1. It failed to develop in laboratory animals in spite of repeated attempts to reproduce it. When the disease was reproduced, the course

was comparable in every way to that of the spontaneous type. Donovan bodies were recovered to the exclusion of other organisms from the pseudobuboes that developed in the 3 patients. The incubation period could not be determined; however, the classic picture of the disease was complete in about fifty days. This is the first instance in which granuloma inguinale was experimentally produced in a human being by the use of an exudate which contained only the Donovan bodies and no other demonstrable organisms.

The pseudobubo that so frequently follows a primary focus on the external genitalia is not adenitis per se but a subcutaneous granuloma. Histologic study of regional and underlying lymph nodes revealed only moderate endothelial hyperplasia. However, of 2 patients Donovan bodies were demonstrated in the underlying cervical and inguinal lymph nodes in one, who also had extragenital involvement, and in one regional inguinal node in the other. Such observations prove that the Donovan body can and does travel by way of the lymphatics. The hypothesis is presented that Donovan bodies may reach the lymph nodes, where temporary though mild focal reactions with perilymphadenitis occur. During this process Donovan bodies may reach the papillae and corium of the overlying skin and set up a subcutaneous granuloma. Here the process may be subacute, resulting in a subcutaneous abscess, or may be chronic and a massive granulomatous tissue causes the overlying epidermis to bulge—hence the pseudobubo, for prior to rupture and the burgeoning of the typical raised granulations it simulates the bubo of the other venereal diseases.

The nature of the Donovan body remains an enigma to most students of the subject. Contrary to the many reports on the isolation and culture of an organism comparable to the Donovan body, it is doubtful whether the causal agent of granuloma inguinale has ever been cultivated. Such cultivated organisms on inoculation into human beings have failed in every instance to reproduce the disease. The method of reproduction in mononuclear endothelial cells and the growth requirements of the organism as well as the clinical behavior of the disease lead the authors to assume that the Donovan body is a sporozoan.

FROM AUTHORS' SUMMARY.

MODE OF ACTION OF SULFANILAMIDE ON STREPTOCOCCUS. F. P. GAY and others, J. Exper. Med. **69**:607, 1939.

The precise mode of the therapeutic action of sulfanilamide on the streptococcus can be arrived at only by considering the sum total of factors that inhibit or favor the natural growth of the micro-organism under the experimental conditions that obtain, whether in vivo or in vitro. Conclusions too sweeping have been drawn from the study of a single variable factor, such as an unfavorable temperature or the absence or the presence of peptone. Gay and his co-workers have attempted here to analyze the factors that have hitherto been recognized and some new ones, but particularly the relationship of these factors to one another. The result obtained on adding sulfanilamide to a culture of the streptococcus in a test tube is usually bacteriostasis and not complete destruction of even small numbers of the bacteria. This is on the condition that the suspending medium is one that is favorable to the growth of the micro-organism; the more growth-promoting the medium is the less the bacteriostasis. If, on the other hand, the medium is too poor or is one that in itself inhibits growth, the addition of sulfanilamide may lead to sterilization of the culture. The conditions for growth of the streptococcus in the body of the rabbit or the mouse depend on the strain of bacteria used, but are on the whole favorable. Defense, however, in the form of phagocytosis by both polymorphonuclear and mononuclear cells is attempted even in the susceptible animal. When sulfanilamide is used to treat such an animal, or when sulfanilamide-grown (inhibited) streptococci are employed, phagocytosis is pronounced, whether studied in the test tube or in the animal body. In the rabbit the delay in streptococcic activity in the presence of sulfanilamide and the resultant increase in phago-

cytosis by polymorphonuclears allow mononuclear cells to accumulate, and recovery may result. Sulfanilamide not only does not completely destroy the streptococcus but does not even impair its innate virulence. It acts on the streptococcus not only by inhibiting growth but by temporarily inhibiting the formation of hemotoxin, but only under certain conditions. The drug does not neutralize hemotoxin already formed. No significant effect of sulfanilamide on the formation of leukocidin or fibrinolysin by the streptococcus has been evident in these experiments. Sulfanilamide differs in one important respect from other drugs that are destructive of protozoa and bacteria either in the test tube or in the body. Protozoa fix or adsorb arsenicals and acriflavine, which kill them variably in vitro and in vivo. Streptococci fix both gentian violet and acriflavine, which have marked destructive action in the test tube but are less effective in vivo. Sulfanilamide is not diminished at all by contact in vitro with large masses of streptococci, nor does its action render these streptococci more capable of absorbing gentian violet or acriflavine than untreated streptococci, to be destroyed by those highly bactericidal substances.

FROM AUTHORS' CONCLUSIONS.

BACTERICIDAL AGENT FROM SOIL BACILLUS. R. J. DUBOS, J. Exper. Med. **70**:1, 11 and 249, 1939.

A gram-positive, spore-bearing aerobic bacillus capable of lysing the living cells of many gram-positive microbial species has been isolated from soil. Cultures of this bacillus in peptone mediums release during autolysis a soluble agent which exerts a bactericidal effect on all the gram-positive micro-organisms so far tested and inactivates their dextrose dehydrogenases. It also inhibits the growth of the susceptible species in culture mediums. Several of the gram-positive species when incubated with the bactericidal agent undergo lysis. It appears, however, that the lysis is only a secondary process, due to the autolytic enzymes of the susceptible cells, and that it follows on some other primary injury caused by the active agent. The bactericidal agent is ineffective against all the gram-negative bacilli so far tested.

Dubos and C. Cattaneo show that the same agent protects white mice against infection with large numbers of virulent pneumococci. It also exerts a curative effect when administered to mice several hours after injection of the infective organisms. The minimal effective dose of the bactericidal agent and the degree of protection afforded are approximately the same for all virulent pneumococci, irrespective of type specificity. The bactericidal agent is entirely ineffective against infection with virulent Friedländer bacilli (type B). This agrees with the fact that the agent does not affect gram-negative bacilli in vitro. The protective action exerted by the bactericidal agent against experimental pneumococcic infection depends on the same mechanism which determines its bactericidal effect in vitro.

A cell-free extract of cultures of an unidentified soil bacillus which exerts a bactericidal effect on gram-positive micro-organisms has been described in previous reports; the first active preparations which were obtained were found to contain a protein precipitable at p_H 4.5. In the present report it is shown that the bactericidal agent can be obtained in an active form free from protein. The purified preparations retain all the activity of the original material, both in vitro and in vivo.

FROM AUTHOR'S SUMMARIES.

A TYPE OF GROUP A HEMOLYTIC STREPTOCOCCUS WHICH FAILS TO FORM PEROXIDE. A. T. FULLER and W. R. MAXTED, Brit. J. Exper. Path. **20**:177, 1939.

Fuller and Maxted note that previous observations on the formation of peroxide by hemolytic streptococci have been without reference to the group or the type of the strain. They show that all known types of hemolytic streptococci of group A form peroxide except type 3. It is remarkable that strains of the latter type, which produce no peroxide in aerobic culture, are inhibited by catalase in the medium.

THE SIZE OF THE VIRUS OF LYMPHOCYTIC CHORIOMENINGITIS AS DETERMINED BY ULTRAFILTRATION AND ULTRACENTRIFUGATION. T. F. M. SCOTT and W. J. ELFORD, Brit. J. Exper. Path. **20**:182, 1939.

Scott and Elford have sought to determine the particle size of the infective agent of lymphocytic choriomeningitis as it exists in broth extracts of infected mouse brain. The evidence furnished by the two methods applied, namely, ultrafiltration and centrifugation analysis, has indicated the probable diameter of the virus to be 40 to 50 microns.

MILIARY TUBERCULOSIS IN THE PANCREAS OF CHILDREN. H. W. SACHS, Frankfurt. Ztschr. f. Path. **51**:63, 1938.

Prompted by Ghon, Sachs examined 20 cases of generalized miliary tuberculosis in order to see if there were changes in the pancreas. The histologic examination by serial sections showed that in 70 to 84 per cent of the cases miliary tubercles were present in the pancreas. Most of the tubercles were found in the peripheral portions of the pancreas and in the head of the pancreas.

OTTO SAPHIR.

LABORATORY CASES OF WEIL'S DISEASE. A. WELCKER, Zentralbl. f. Bakt. (Abt. 1) **141**:400, 1938.

Welcker discusses the problem of laboratory infections by Leptospira icterohaemorrhagiae. He summarizes the literature dealing with such accidents and cites numerous reports of cases in which workers have inoculated themselves accidentally with a syringe or have been bitten by rats. He also emphasizes the fact that within recent years these spirochetes have been found in healthy white rats and that several cases of infection from the handling of these animals have been reported. No original data are given inasmuch as the paper is essentially a summary of the problem and is meant to serve as a warning to workers who may run the danger of becoming infected while handling white rats.

PAUL R. CANNON.

RÔLE OF VITAMIN C IN THE GENESIS OF TUBERCULOSIS IN THE GUINEA PIG. K. E. BIRKHAUG, Acta tuberc. Scandinav. **13**:45 and 52, 1939.

Daily oral administration of crystalline vitamin C significantly inhibits the tuberculin reaction in tuberculous guinea pigs. The degree of inhibition is definitely correlated with the urinary excretion and adrenal content of vitamin C.

Hypervitaminosis C induced by daily oral administration of 10 mg. of l-ascorbic acid caused a significant increase in body weight and reduction in the invasive lesions and development of generalized tuberculosis in guinea pigs which had been inoculated subcutaneously with approximately 500 viable bovine tubercle bacilli each and killed sixty-four days after inoculation.

A histologic study revealed less of caseonecrotic lesions, more collagenous tissue within and around the tuberculous centers and less dispersion of tubercle bacilli in animals with hypervitaminosis C than in the controls.

FROM AUTHOR'S SUMMARIES.

Immunology

ANTIGENICITY OF THE VIRUS OF TRACHOMA. L. A. JULIANELLE, Am. J. Path. **15**:279, 1939.

Clinical observation reveals little if any immunity to trachoma. It has not been possible to demonstrate increased resistance to experimental trachoma in

monkeys following recovery from the infection. The serum or defibrinated blood of patients with active infections of varying duration exerts no neutralizing or protective effect on the virus of trachoma. The serum of infected or recovered monkeys contains no antibodies demonstrable by the usual methods of protection. The serum of rabbits or susceptible monkeys receiving repeated intravenous injections of active trachomatous tissues contains similarly no antiviral substances. It is concluded that the virus of trachoma is an impotent and ineffectual antigen.

FROM AUTHOR'S SUMMARY.

SEROLOGICAL SPECIFICITY OF PEPTIDES. K. LANDSTEINER and J. VAN DER SCHEER, J. Exper. Med. **69**:705, 1939.

Experiments are described dealing with immune serums to pentapeptides and peptide amides. Absorption and inhibition tests gave no indication of the presence in the immune serums of special antibodies for portions of a peptide molecule, but the antibodies appeared to be specific for an entire pentapeptide even though the serums contained qualitatively different fractions. Marked disparity was found between the reactions of peptides and corresponding amides, indicating differences between acid and other polar groups in their influence on serologic specificity.

FROM AUTHORS' SUMMARY.

SENSITIZATION WITH CHEMICAL COMPOUNDS. K. LANDSTEINER and M. W. CHASE, J. Exper. Med. **69**:767-1939.

Experiments are described on the period of latency in sensitization to poison ivy and on the time the agent must remain in contact with the skin. The chief matter of investigation concerned the manner in which the whole skin becomes sensitive following treatment at a particular site, especially whether this sensitization is effected by way of the epidermis. Two methods were used to interrupt the continuity of the skin, one that of cutting through both the skin and the underlying thin muscular layer, the other that of removing a strip of skin so as to spare the cutaneous muscle. These procedures led to different results when an extract of poison ivy was applied to the areas thus isolated. With the first method sensitization was mostly prevented, whereas with the second method generalized hypersensitiveness occurred almost uniformly. An explanation is to be found in the severance of the lymph vessels lying on the surface of the muscular layer, pointing to the necessity of a free passage of lymph. On the other hand, the experiments prove that general sensitization is not dependent on maintaining the integrity of the skin around a treated area. An inhibition of sensitization by incisions extending through the panniculus carnosus was seen to some extent in anaphylactic sensitization with protein antigens, namely, when sufficiently small amounts were employed.

FROM AUTHORS' SUMMARY.

SEROLOGICAL STUDIES OF REPTILIA. G. C. BOND and N. P. SHERWOOD, J. Immunol. **36**:1, 11, 1939.

Isohemagglutination was not observed, but heterohemagglutination and heterohemolysis occurred between genera and between families of the Serpentes, but they showed no relation to zoologic grouping. Snake red blood cells did not contain agglutinogens comparable to human A and B, but some snake serums contained species-specific, and a few contained group-specific, agglutinins capable of reacting with human red cells. These serums when properly absorbed can be used reliably to determine the blood groups of human red cells. Each of 38 specimens of undiluted fresh snake serum hemolyzed both human and sheep red cells, the titer for the latter being considerably higher. The hemolytic action was found to be due to a normal hemolysin and to an excess of complement, which had

a titer, and which reacted to heat and in storage, similarly to guinea pig complement. Snake complement could be used in bacterial and in syphilitic complement fixation tests. That it was not identical with guinea pig complement was demonstrated by the fact that the latter could not take its place in the hemolysis of sheep red cells by strongly hemolytic snake serums. I. DAVIDSOHN.

NONSPECIFIC "DESENSITIZATION" THROUGH HISTAMINE. L. FARMER, J. Immunol. **36**:37, 1939.

Young virgin guinea pigs were sensitized with horse serum and after from thirteen to fifteen days were given repeated intra-abdominal injections of histamine in increasing amounts. From twenty-six to thirty-six days after sensitization their uteri were tested in the Schultz-Dale experiment. Much larger amounts of horse serum were needed to bring about anaphylactic contraction (with 78 per cent a concentration of more than 1:250,000) in these uteri than in controls, with which the concentration needed was only 35 per cent. Injections of histamine into 9 guinea pigs caused them to become less sensitive to the same substance. The results suggest that histamine is the substance responible for the anaphylactic contraction of the smooth muscle and thus they support Dale's hypothesis.

I. DAVIDSOHN.

SERUM SICKNESS IN RABBITS. M. S. FLEISHER and L. R. JONES, J. Immunol. **36**:511, 1939.

Horse immune serum to which proper amounts of sodium hydroxide had been added was heated at an optimum temperature for established periods of time and then neutralized with hydrochloric acid. The product retained the major part of the antibody but failed to produce manifestations of serum disease in rabbits. A definite quantitative interdependence was observed among the three factors: alkali, temperature and time of heating. There is evidence to the effect that concentrated pseudoglobulin can be similarly treated, effecting destruction of the factor causing serum sickness without excessively injuring the antibody. However, the toxic manifestations that were observed in some rabbits prohibit at present the application of this method of eliminating the factor causing serum sickness preliminary to the use of the antitoxin in man. I. DAVIDSOHN.

PROPERTIES OF ANTIGENIC PREPARATIONS FROM BRUCELLA MELITENSIS. A. A. MILES and N. W. PIRIE, Brit. J. Exper. Path. **20**:109, 1939.

Miles and Pirie describe the serologic behavior of the antigen of Brucella melitensis in its native state and after stepwise degradation by various agents. The relationship between the physical properties of a solution of the antigen and the character of the serum precipitate is discussed.

ACTION OF PROTEOLYTIC ENZYMES ON THE ANTITOXINS AND PROTEINS IN IMMUNE SERUM. C. G. POPE, Brit. J. Exper. Path. **20**:132, 1939.

Pope has reexamined the possibility of purifying antitoxic serum by digestion of inactive protein with proteolytic enzymes (mainly pepsin). Working with diphtheria antitoxic serum, he has determined the conditions necessary for maximum digestion of inactive protein and minimum loss of antitoxic activity. Samples of plasma from different horses show considerable variation in response to pepsin action; usually plasma from horses which have responded well to immunization give the best results. It is concluded that peptic digestion alone does not provide a suitable method for the purification of antitoxin since the degree of purification is rather low (up to four times) and the products of the breakdown of inactive protein are difficult to separate from the antitoxin.

CULTIVATION OF THE VIRUS OF AUJESZKY'S DISEASE ON THE CHORIOALLANTOIC MEMBRANE OF THE DEVELOPING EGG. R. E. GLOVER, Brit. J. Exper. Path. **20**:150, 1939.

No effective method has yet been devised for inducing satisfactory immunity against Aujeszky's virus disease of animals (pseudorabies). Glover has endeavored to adapt the virus to the developing egg in the hope that the infective agent would undergo mutation and serve as an immunizing agent. The virus has been successfully propagated on the egg. Some evidence of mutation has been obtained, but the virus is not yet sufficiently attenuated to permit employment of it for purposes of vaccination.

Tumors

CEREBELLAR ASTROCYTOMA. P. C. BUCY and W. A. GUSTAFSON, Am. J. Cancer **35**:327, 1939.

Cerebellar astrocytomas occur for the most part in children. They are well circumscribed solid or cystic gliomas, which can usually be readily and successfully enucleated. They are composed predominantly of fibrillary and protoplasmic astrocytes in variable proportions, in association with a very small percentage of other adult and embryonic cells of the spongioblastic series. They contain no ganglion cells or nerve fibers other than those engulfed as a result of their invasion of the cerebellum and no neuroblasts. They not infrequently invade the subarachnoid space, and in such areas one may find glial bridges connecting the molecular layer of the cerebellum and the subarachnoid space. Degenerative changes involving the cells of the tumor, their processes and fibrillae and the blood vessels are common and are not infrequently misinterpreted by the unwary. The surrounding cerebellum shows the effects of compression and ischemia. Although it is possible that many cerebellar astrocytomas are congenital, arising from a developmental fault, this hypothesis remains unproved. There is no evidence to support the contention that these are congenital malformations rather than neoplasms. The small intraneoplastic cysts which develop are the result of liquefaction of the tumor tissue, but the extraneoplastic cysts, and perhaps the large intraneoplastic ones as well, appear to be formed by transudation. The original classification of these tumors into fibrillary and protoplasmic astrocytomas is accurate and valuable. More recent efforts at alteration of the classification or of its nomenclature are illogical, of little value and confusing.

FROM AUTHORS' SUMMARY.

THE PRODUCTION OF CARCINOMA AND SARCOMA IN GUINEA-PIGS BY THOROTRAST [COLLOIDAL THORIUM DIOXIDE]. L. FOULDS, Am. J. Cancer **35**:363, 1939.

Four injections of 0.2 to 0.3 cc. of colloidal thorium dioxide made into the base of a nipple produced tumors in 4 of 9 guinea pigs which survived until the earliest growth was detected. The average induction time was about thirty-seven months. The tumors comprised 1 carcinoma, 2 sarcomas and 1 fibrosarcoma; the carcinoma and the sarcomas were transplantable. The carcinoma has been transmitted through fifteen generations. The experiment demonstrated the production of a carcinoma other than a squamous carcinoma by the local action of a carcinogenic agent.

FROM AUTHOR'S SUMMARY.

IMMUNITY TO SHOPE FIBROMA VIRUS. J. CLEMMESEN, Am. J. Cancer **35**:378, 1939.

Shope fibroma virus injected into rabbits treated by general roentgen irradiation produces results similar to those described by Andrewes and others in rabbits given injections of tar, namely, prolonged growth of the resultant fibromas, prolonged resorption and general fibromatosis, after intravenous inoculation. The development of immunity to repeated inoculations with this virus is delayed in roentgen irradiated rabbits. The effects of general roentgen irradiation on transplanted tumor and normal cells, on inoculated fibroma virus and artificial carcino-

genesis have their parallels in the effects of injections of tar and of trypan blue. It is suggested that all these procedures act through the reticuloendothelial system.

FROM AUTHOR'S SUMMARY.

EFFECT OF OIL OF WINTERGREEN ON SPONTANEOUS TUMORS. L. C. STRONG, Am. J. Cancer **35**:401, 1939.

Extensive liquefaction of spontaneous tumors of the mammary glands in mice may be brought about by introducing heptylaldehyde into the organism through the diet. Liquefaction of spontaneous tumors may also be produced by injecting heptylaldehyde subcutaneously in areas remote from the neoplasm. Because of hemorrhage from the surface of the tumor and absorption of possible toixc agents from large or advanced growths, great caution should be employed in the use of heptylaldehyde or of any other agent that has a similar action on spontaneous tumors. The synergistic or tandem use of two or more chemicals may be the means of controlling spontaneous tumors by chemotherapy, at least in mice.

FROM AUTHOR'S SUMMARY.

CONTORTED MITOSIS AND THE SUPERFICIAL PLASMAGEL LAYER. W. H. LEWIS, Am. J. Cancer **35**:408, 1939.

The assumption that cells have a superficial layer of gelated cytoplasm (plasmagel layer) which automatically exerts continuous contractile tension and that this layer undergoes various local and general changes in viscosity and thickness, with corresponding variations in its contractile tension, offers a key to one of the important factors concerned in changes of cell form, in cell locomotion and in cell division. The contorted mitoses observed in the division of the cells of spindle cell sarcoma C37 are explained by the development of changing contraction bands of the plasmagel layer which produce constrictions that result in marked distortions and lobulations of the dividing and young daughter cells.

FROM AUTHOR'S SUMMARY.

EXPERIMENTAL ADENOMA OF THE PITUITARY. I. H. PERRY and M. S. LOCKHEAD, Am. J. Cancer **35**:422, 1939.

Three pituitary tumors developed in 131 female mice receiving prolonged treatment with estrone (theelin); none was present in 97 controls. Two of these mice also had inguinal implants of 1,2,5,6-dibenzanthracene.

FROM AUTHORS' SUMMARY.

PULMONARY ADENOMATOSIS (JAGZIEKTE) IN SHEEP. C. BONNE, Am. J. Cancer **35**:491, 1939.

In the human lung a diffuse tumor-like condition, here described as carcinosis, may occasionally be found. Morphologically, this closely resembles certain pulmonary diseases of animals, of which jagziekte in sheep is the best known representative. In these diseases the alveolar cells and sometimes the bronchiolar cells are replaced by dark-staining cells in which mitoses are frequent. These cells line the alveolar sacs and often protrude into their lumens, forming papillomatous buds. There is practically no invasive growth, and metastases are absent. The possibility of a virus origin of these diseases is discussed. A detailed description is given of a case of general pulmonary carcinosis in a Chinese.

FROM AUTHOR'S SUMMARY.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

S. A. Levinson, *President*

Edwin F. Hirsch, *Secretary*

Regular Monthly Meeting, Oct. 9, 1939

Presidential Address: I. The History and Progress of the Scientific Work of the Cook County Coroner's Office. Samuel A. Levinson.

The coroner's office in Cook County was established in 1864. Ernst Schmidt, the first coroner of Cook County and the first medical man to hold this position, was elected on the Republican ticket at this time.

Schmidt, who was a liberal and an exponent in the defense of civil and political liberties, graduated from the University of Wurzburg in 1857 and arrived in Chicago in the same year. During his student days in the University of Wurzburg he was active in the revolutionary movement in the year 1848-1849. There he later attended the lectures of Virchow and became his enthusiastic disciple and friend. Schmidt and Virchow had a common bond in that both were interested in social and political reforms. When Schmidt arrived in the United States, and later in Chicago, he carried on these reforms along the lines of a defender of civil rights and liberties, and among other activities he "stumped" the state of Illinois for Abraham Lincoln for President of the United States. One outstanding example of the work performed by Schmidt was his active part in the defense of the "Haymarket rioters" in Chicago in 1887.

Schmidt aided in the founding of the Humboldt College of Medicine in St. Louis, and at the outbreak of the Civil War he joined the third Missouri brigade and was with this unit until illness forced him to retire. On his return to Chicago in 1864 Schmidt was elected the first coroner of Cook County. He was regarded as one of the first scientific men in medicine, as well as one who had received training in pathology under Rudolf Virchow. Schmidt was coroner of Cook County for a little over a year, when he resigned because of his inability to cope with political influences in his office. He was interested in making anatomic dissections and postmortem studies in the various cases of death referred to his office, but was unable to carry on the scientific work.

In 1890 Henry Hertz was elected coroner of Cook County, and he appointed the first coroner's physician—Ludvig Hektoen.

Hektoen's experiences as coroner's physician during this time are comparable to the experiences of most physicians who covered large territories with horse and buggy. Hektoen's work included investigations of deaths due to natural causes, and he issued death certificates. His duties also consisted of making autopsies at the Cook County Hospital for the coroner, as well as investigating causes of deaths and making autopsies in the various undertaking rooms in Chicago and Cook County. There were no streetcars or elevated lines extending to the suburban areas, and the horse and buggy method of conveyance took considerable time. Because of the large area that had to be covered, Hektoen could not always complete the work assigned each day. Aside from duties as coroner's physician he was called on to testify in criminal cases, which took a great deal of time. He was also professor of pathology at the College of Physicians and Surgeons. Hektoen could not keep detailed records of all the autopsies performed during this time, but he did make use of the interesting pathologic material that presented

itself. There were no medical libraries available; the literature was scanty, and he was pressed for time to study in detail the material he wished to report. A review of the pathologic material presented before the Chicago Pathological Society at its beginning reveals an amazingly large number of studies made by Hektoen on gross as well as on histologic material which he had obtained as coroner's physician. More than forty articles were published during the time that he held this position.

Hektoen, as first coroner's physician, started a system which he called "investigation record," giving the facts as he found them at the autopsy table. He urged strongly that records of observations made at autopsy were of great importance to the coroner's office for their legal as well as scientific value.

Louis J. Mitchell, who had started his internship at the Cook County Hospital six months before Hektoen, was the second coroner's physician in Cook County and served for four years. One of the important autopsies performed by Mitchell, who had called Hektoen in as consultant, was that on the body of Mayor Carter Harrison, who had been assassinated by Prendegast.

When Peter M. Hoffman was elected coroner of Cook County in 1912, he organized an advisory committee to recommend competent candidates for coroner's physicians as well as to advise on other matters. E. R. LeCount was appointed coroner's physician and was stationed at the Cook County Hospital Morgue.

Hektoen recently wrote the following concerning LeCount:

"He set a high standard of necropsy and of necropsy records. In connection with his medicolegal work he developed a well organized system of volunteer assistantships. The duties were indeed arduous but the opportunities to learn were so attractive that there never was any lack of acceptable applicants. The work began between four and five o'clock in the morning so that it might be finished before the hour of the regular course in pathologic anatomy. So tireless was LeCount's industry, so fully had he taken himself in hand, that during these years he rose shortly before four o'clock. In the course of his medicolegal service he gathered a remarkable series of records of cases (99 volumes, fully indexed), which form a copious source of information concerning the pathologic anatomy of medicolegal conditions. He was engaged in the study of this material to the last, and in his room in the hospital he completed valuable analyses of cases of fractures of the skull and of gunshot wounds involving the chest and abdomen simultaneously.

"As the years passed he developed into an authority in practical pathology, clinical and medicolegal. His experience, the skill and thoroughness of his examinations, his wide knowledge, his refusal to come to any final decision in the absence of conclusive evidence, and the soundness of his judgments gave him the standing of a supreme judge in questions of diagnosis on a structural basis."

From 1923 to 1927 Oscar Wolff was coroner of Cook County and we can refer to this era only as the "four dark years."

As a result of Wolff's administration as coroner of Cook County, the law-abiding citizens of Chicago and Cook County made a protest against the method and conduct of the coroner and drafted Herman M. Bundesen as a candidate to oppose Wolff. Bundesen was elected by an overwhelming majority.

Bundesen assumed office in 1928 and resigned in 1931 to become health commissioner. During his tenure in office he introduced numerous reforms, reorganized his staff of pathologists and elevated the scientific division of the coroner's office to its present high level. He also appointed an advisory committee consisting of Ludvig Hektoen, J. P. Simonds, W. F. Petersen, Frank McJunkin and Louis Schmidt.

F. J. Walsh, the present coroner of Cook County, has continued along the lines recommended by his medical advisory committee and has at no time interfered with the scientific and professional work of his physicians and toxicologist. He has maintained his scientific staff at its present high level.

W. D. McNally was appointed first coroner's chemist, and Ralph Webster served for about one year. C. W. Muehlberger is the present coroner's toxicologist.

II. Analysis of the Work Done by the Scientific Staff of the Cook County Coroner's Office.

The percentage of all deaths occurring in Cook County which are investigated by the coroner's office varies from 12.73 to 21.63. This percentage is much higher than has heretofore been reported by various analysts. The total number of cases investigated by the coroner's office varied from 4,937 in the year 1909 to 9,011 in the year 1928.

The number of autopsies performed by coroner's physicians during the years 1931 to 1938 is also much higher than has previously been reported. The percentage of all deaths due to natural causes as well as deaths resulting from trauma or violence on which autopsies were done varied from 17.32 in 1932 to 26.4 in 1935. The percentage of deaths investigated by the coroner's office on which autopsies were done compares favorably with that of similar offices in the United States, and it certainly compares favorably with that in most of the hospitals of Chicago.

An analysis was made of the number of deaths resulting from homicide, suicide, poisoning and abortions. The highest level of deaths due to homicide was in 1929, during the prohibition era. When the Prohibition Act was repealed by Congress, in 1933, there was a sudden drop in the number of homicides.

Deaths due to suicide had two peaks, one in 1915, during the World War, and another in 1929-1930, during the economic crash. The economic crash was not the sole factor in the increase in the number of suicides in 1929-1930, and a more detailed analysis of this will be reported elsewhere.

A similar analysis was made of the number of deaths due to poisons as well as of the number due to abortion.

In 1929 the firearms investigation bureau was established in Chicago. The coroner's office has a ballistics unit which is an integral part of this office. The firearms identification bureau examined as many as 1,431 guns in the year 1932. In 1930 647 bodies dead from bullet wounds were submitted to autopsy, and 1,131 bullets were recovered and given ballistics examination. The toxicologic department, which is an integral part of the coroner's office, examines not only material submitted from the bodies dead from or suspected of being dead from poisoning but medicines and other chemicals found on the dead body or in the vicinity of the dead body. For example, in 1929 there were 994 bodies dead from or suspected of being dead from poisoning, and 1,277 examinations were made for poisons and on material submitted by the Cook County Purchasing Department.

The material obtained at coroner's autopsies differs in many respects from that obtained at "permission" autopsies. It includes specimens representative of unexpected or sudden deaths, as well as deaths from poisoning, drowning, electrocution, abortion, bullet wounds, industrial and transportation accidents, etc. The material is utilized for teaching purposes as well as for exhibits.

A course for the teaching of legal medicine in medical schools has been organized in the past few years.

The scientific work performed by the coroner's office should form a nucleus for the establishment of an institute of legal medicine in Cook County. The crime detection laboratory, the department of police administration, the institute for juvenile research and the psychiatric institute, and the firearms identification bureau are all services which are directly or indirectly extensions of the pathologic and toxicologic departments of the coroner's office. These can be utilized in aiding the courts and prosecution as well as the defense in the conduct of legal cases. Although they function independently, they aid one another in a cooperative way. If this loose integration could be organized into a close corporation and associated with the legal group, a true type of medicolegal institute would be approached which would be of great service to the citizens of our community.

OCCURRENCE AND SIGNIFICANCE OF CONGENITAL MALIGNANT NEOPLASMS.
H. GIDEON WELLS.

Human cancer when produced by known agencies (e. g., roentgen rays, radium, or chemicals as in dye workers' cancer) has been found usually to require seven to fifteen years for its development. Experimental study of cancerogenesis has shown that such a long total period of development is not necessary but that what is necessary is a comparable part of the normal life span. Hence experimental cancer appears quicker in mice than in rabbits, in rabbits than in dogs, in dogs than in man. In striking contrast to this general principle is the fact that there is a group of tumors that usually appear early in life or not at all, outstanding of which are the neuroblastoma of the retina, the neuroblastoma of the adrenal gland and the malignant nephroma. A study of cases of these tumors shows that in a considerable proportion they are already present as malignant neoplasms at the time of birth. In our laboratories, in about 3,000 necropsies on fetuses born dead or on infants dying shortly after birth there were observed 3 instances of congenital adrenal neuroblastoma and 1 instance of neuroblastomatous involvement of much of the sympathetic nervous tissue.

Summary of Recorded Congenitally Malignant Tumors (Not Including Retinal Tumors)

	Accepted	Probable	Possible	Total
Malignant renal tumor	5	11	?	16
Malignant adrenal neuroblastoma	17	15	21	53
Malignant extra-adrenal neuroblastoma	6	4	?	10
Congenital sarcoma	33	29	53	115
Teratoma with malignant character at birth	1	2	?	3
Tumors of undetermined nature	3	1	7	11
Carcinoma of the liver	0	1	9	10
Hemangioendothelioma malignum of the liver	?	?	15	15
Tumor of the liver of undetermined character	?	?	4	4
Carcinoma, excluding liver	0	0	5	5
Cerebral glioma	1	1	2	4
Malignant endothelioma, excluding liver	?	?	5	5
Melanoma malignum	0	2	2	4
	66	66	123	255

In the hope that a study of cases of malignant growth present at birth might offer some suggestions as to the meaning of this deviation from the usual requirements for malignant neoplasia, a review of the literature has been made, and many misinterpretations have been corrected. The results are summarized in the accompanying table.

There have been no congenital carcinomas of the types commonly seen in adults which were indisputably established to be such, although a few lesions reported as congenital carcinoma of the liver or thymus may be genuine. On the other hand, there are not a few unquestionably congenital sarcomas which seem to differ in no noteworthy respect from the sarcomas of adults. Since it requires about the same length of time to produce experimental sarcomas and carcinomas, the explanation for this difference is not clear.

While a large proportion of neurogenic malignant tumors derived from the tissues of the sympathetic nervous system and from the retina, when present, are present at birth, congenital neoplasms of the brain and peripheral nerves are extremely rare. Evidence is found that congenital maldevelopments of nerve tissues occur frequently in brain, retina and adrenal, and these apparently may disappear, develop into malignant tumors, develop into benign tumors or even become malignant and then change in whole or in part into nonmalignant tissues.

In striking contrast to retinoblastoma in which there is a marked hereditary factor, the other types of tumor tend to be congenital or, if they appear in early infancy, show no familial or hereditary influences.

Placental tumors, occurring as they do in fetal tissues, may not be an exception to the rule that from one fifth to one tenth of the life cycle of an organism ordinarily is required to produce a malignant growth, since the placenta is an organism the entire life cycle of which terminates in senility in about six months.

Some cases of true congenital leukemia have been reported, but not a single instance has been found in which the leukemia was transmitted from the mother to the fetus, although there have been a few instances of metastasis of maternal cancer to the fetus.

The significance of congenital malignant growth is discussed in the light of the fact that ordinarily a large part of the life cycle is required to produce any type of malignant tumor.

Presumably the malignant growths which appear chiefly before or soon after birth depend on some other mechanism or principle than do the ordinary malignant growths that require so long for their production. Cramer has directed attention to the fact that the retinoblastoma, which appears almost exclusively in infants, and the chorionepithelioma arise from tissues that have a type of metabolism resembling that of tumors. Besides the retinoblastoma, the neuroblastoma and the nephroblastoma are also tumors of the first few years of life, and in recent years it has been shown that in the brain and the kidney medulla glycolysis is high. Thus these tissues also have a metabolism resembling that of the embryo and of tumors, and this may have some connection with the occurrence of these tumors in the early years of life.

DISCUSSION

S. R. ROSENTHAL: I have seen a number of swine with Wilms' tumor of the kidney in a benign form. The tumor usually occupies one pole of the kidney. It sometimes becomes very large but never metastasizes. Histologically, there are rudimentary tubules and glomeruli in a matrix of cellular connective tissue.

TUMORS OF THE SYMPATHETIC NERVOUS SYSTEM. EDITH L. POTTER.

Neuroblastoma and ganglioneuroma are tumors composed of cells representing different degrees in the development of primitive tissue arising originally from the neural crest of the embryo. The structure varies from that of tumors which are composed entirely of completely undifferentiated cells (sympathogonia), moderately differentiated cells (sympathoblasts) or completely differentiated cells (ganglion cells) to that of tumors in which there is a mixture of all elements. Schwannoma has been found in association with chromaffin tumors but only rarely in association with neuroblastoma or ganglioneuroma.

The present case is that of an infant born dead and two months prematurely, in whom multiple tumors were present. The entire chain of paravertebral sympathetic ganglions was transformed into a continuous mass of tumor tissue, 1 to 1.5 cm. in diameter. Fused with the inferior surface of each adrenal were lobulated, circumscribed tumors, each 5 by 5 by 6 cm. Connecting these across the midline was a similar tumor of approximately equal size. The wall of the urinary bladder was firmer, more fibrous tumor tissue, 3 cm. in maximum thickness. It extended posteriorly and encircled the rectum, the fibers ending at the anus. Numerous small encapsulated masses of tumor tissue were in the posterior part of the abdominal cavity. There was extreme hypertrophy of all nerves in the body, particularly of those of sympathetic origin. Multiple minute tumor nodules were present throughout the liver.

Microscopically, all of the tumors had a similarity in structure and were composed of sympathogonia, sympathoblasts, ganglion cells and nerve fibers, with irregular regions of hemorrhage, calcification and necrosis. Sympathogonia formed the main portion of the adrenal tumors, ganglion cells and fibers composed the greater part of the ganglion tumors, but all types formed a diffuse mixture in all tumors.

The tumor of the bladder differed from the tumors in other locations. It had large masses of tissue composed of elongated nuclei and fibrillar material separated

into bundles of collagen fibers. In one region groups of immature ganglion cells were present, but most of the tumor consisted only of Schwann cells and fibers, characteristic of schwannoma. The hypertrophy of the nerves was due to an increase in Schwann cells accompanied by local excessive proliferation of acellular fibrils.

This case exemplifies the interrelationship of neuroblastoma, ganglioneuroma and schwannoma. The tumor is due not to activation of misplaced rests of embryonic tissue but to some condition affecting the entire sympathetic nervous system, stimulating generalized neoplastic growths.

INTRACAPILLARY MICROSCOPIC METASTATIC MAMMARY GLAND CARCINOMA OF THE LUNGS AND OTHER VISCERA. LESLIE R. GRAMS.

This report was published in full in the December issue of the ARCHIVES, page 865.

Book Reviews

Medical Jurisprudence and Toxicology. William D. McNally, A.B., M.D., Assistant Professor of Medicine and Lecturer in Toxicology, Rush Medical College, University of Chicago. Pp. 386. Price $3.75. Philadelphia: W. B. Saunders Company, 1939.

The book is divided into two sections: part 1, "Medical Jurisprudence," 69 pages; part 2, "Toxicology," 259 pages. Part 1, which is supposedly written primarily for the physician and the medical student, is entirely inadequate and the treatment rather superficial. About a ninth of the book has been allotted to the topic of medical jurisprudence, and essential phases have been omitted, or, if mentioned, have not been described in sufficient detail; for example, the matter of the signs of death, an important medicolegal problem, has been disposed of in 3 pages; sudden and unexpected deaths from natural causes, which on the average constitute more than 50 per cent of the total number of deaths that come to the attention of the coroner or the medical examiner, have been allotted but 2 pages; injuries, burns, abortions, infanticides and insanity are lumped together and rather inadequately treated in the space of 25 pages; bullet wounds, stab wounds and rape are mentioned very briefly, while, in contrast, ballistics, a matter primarily of concern to the police, has been given 9 pages! A further criticism of this section is that numerous careless and misleading statements are made which will raise doubts in the mind of any discriminating reader. One might mention particularly the section on blood grouping, especially the paternity table on page 71, in which 12 errors have been made. Any one using this table would be grossly misled.

In the second part of the book, that on toxicology, the symptoms of poisoning and the treatment of various types of poisoning are given in a concise form and are well presented. However, the discussion of the detection and the quantitative determination of the poisons that may be present in tissues is fragmentary and indefinite. In the description of the methods abrupt transitions and poor sequences leave much to be desired. An inexperienced analyst attempting to carry out an analysis of organs for poisons by following the directions in the text could not hope for much success; an experienced analyst would not be apt to use the book.

Many important laboratory procedures are scantily treated, only general principles being outlined. Some of the more recent and specific tests of importance have not been mentioned. Methods of isolation and purification of toxic principles, which must precede the application of identification tests, have in the case of several of the poisons been totally ignored. Crystal tests for several of the alkaloids do not appear. Concerning the chemical procedures many ambiguous and erroneous statements are made.

One realizes that it is impossible to confine in a volume of this size detailed descriptions of all drugs and chemicals that may enter into suicides, homicides and accidental poisonings, but one does feel that if any tests are given they should be so stated that a person with the necessary fundamental training can carry them out successfully. This the book does not do, and therefore it cannot be considered as satisfactory to serve as a textbook on toxicology.

Books Received

COMMUNICATIONS DU LABORATOIRE BACTERIOLOGIQUE DE L'ÉTAT SUÉDOIS. Volume XII, 1937. Paper. Various pagination. Uppsala: Almqvist & Wiksells Boktryckeri-A.B., 1939.

REPORT OF THE PRESIDENT AND OF THE TREASURER FOR THE YEAR ENDED SEPTEMBER 30, 1939, CARNEGIE CORPORATION OF NEW YORK. Paper. Pp. 167. New York: Carnegie Corporation of New York, 1939.

PROCTOSCOPIC EXAMINATION AND DIAGNOSIS AND TREATMENT OF DIARRHEA. M. H. Streicher, M.S., M.D., University of Illinois College of Medicine, Chicago. Cloth. Pp. 149, with 39 illustrations. Price $3. Springfield, Ill.: Charles C. Thomas, Publisher, 1940.

REPORTS ON BIOLOGICAL STANDARDS—V: VARIABLES AFFECTING THE ESTIMATION OF ANDROGENIC AND OESTROGENIC ACTIVITY. C. W. Emmens. Medical Research Council, Special Report Series, no. 234. Paper. Pp. 71. Price 40 cents; 1 shilling, three pence. New York: British Library of Information. London: His Majesty's Stationery Office, 1939.

A SYMPOSIUM ON THE BLOOD AND BLOOD-FORMING ORGANS. E. Meulengracht, Cecil James Watson, C. P. Rhoads, Clark W. Heath, George R. Minot, Louis K. Diamond, Russell L. Haden, J. Furth, Claude E. Forkner, E. B. Krumbhaar, Charles A. Doan, Hal Downey, Paul Reznikoff, Edwin E. Osgood and Harry Eagle. Cloth. Pp. 264, illustrated. Price $3.50. Madison: The University of Wisconsin Press, 1939.

THE RISE OF EMBRYOLOGY. Arthur William Meyer, Professor of Anatomy, Emeritus, Stanford University. Cloth. Pp. 367, with 97 illustrations. Price $6. Stanford University, Calif.: Stanford University Press, 1939.

CLINIQUE ET PHYSIOPATHOLOGIE DES MALADIES COELIAQUES. Robert Dubois, Agrégé de l'enseignement secondaire, Médecin-adjoint de la Clinique infantile de l'Université de Bruxelles. Preface du professeur E. J. Bigwood. Paper. Pp. 350, with 14 illustrations. Price 80 francs. Paris: Masson & Cie, 1939.